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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 February 1998 (25.02.98)	
International application No. PCT/NL97/00345	**Applicant's or agent's file reference** PCT 0589
International filing date (day/month/year) 19 June 1997 (19.06.97)	**Priority date** (day/month/year) 20 June 1996 (20.06.96)
Applicant VAN LEENGOED, Leonardus, Andrianus, Maria, Govardus et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   20 January 1998 (20.01.98)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

R. Raissi

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PCT 0589		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 97/00345	19/06/1997	20/06/1996
Applicant		
KOSTER, Henk W. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant.
   - [X] the text has been established by this Authority to read as follows:

   IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL 97/00345

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claim(s) 18 and 19 (both partially, as far as an in vivo method is concerned) are directed to a diagnostic method practised on the human/animal body, and although claims 15 and 22 (both completely) and claim 16 (partially, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00345

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/54 C07K14/715 C07K16/24 C07K16/28 C07K16/42
A61K38/17 A61K38/20 A61K39/395 G01N33/68 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9607 Derwent Publications Ltd., London, GB; AN 96-065477 XP002027134 & JP 07 324 098 A (DAICEL CHEM. IND. LTD. ET AL.) , 12 December 1995 see abstract ---	1,3,6, 14-16
X	DATABASE WPI Week 9607 Derwent Publications Ltd., London, GB; AN 96-065476 XP002027135 & JP 07 324 097 A (DAICEL CHEM. IND. LTD. ET AL.) , 12 December 1995 see abstract --- -/--	1-6, 14-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1997	10. 11. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9035 Derwent Publications Ltd., London, GB; AN 90-266224 XP002027136 & JP 02 188 600 A (CHUGAI PHARMACEUTICAL KK) , 24 July 1990 see abstract	1,3,6, 14-18
X	WO 95 04075 A (MEDVET SCIENCE PTY. LTD.) 9 February 1995 see examples 15,16 see seq. id. nos 17,36,37 see table 2	1-7, 14-16,21
X	EP 0 426 857 A (KURARAY CO. LTD.) 15 May 1991 see claim 7	2,3,6,7, 16
X	US 5 210 075 A (SCHOLZ ET AL.) 11 May 1993 see examples	1-5,12, 14-16
X	C. MORTON ET AL.: "Solution structure of synthetic peptides corresponding to the C-terminal helix of interleukin-6." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 219, no. 1-2, 15 January 1994, BERLIN, GERMANY, pages 94-107, XP000645297 see page 98, left-hand column, line 50 - line 61 see page 105, right-hand column, line 20 - page 106, left-hand column, line 4	1,3,4,6, 7
A	M. KALAI ET AL.: "Participation of two Ser-Ser-Phe-Tyr repeats in interleukin-6 (IL-6) binding sites of the human IL-6 receptor." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 238, no. 3, June 1996, BERLIN, GERMANY, pages 714-723, XP000645294 see table 1	12

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 97/00345

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504075 A	09-02-95	AU 7341494 A	28-02-95
		CA 2168261 A	09-02-95
		EP 0715633 A	12-06-96
		JP 9501154 T	04-02-97
EP 426857 A	15-05-91	CA 2026881 A	09-08-90
		WO 9009396 A	23-08-90
		US 5171837 A	15-12-92
US 5210075 A	11-05-93	NONE	

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/54, 14/715, 16/24, 16/28, 16/42, A61K 38/17, 38/20, 39/395, G01N 33/68, 33/577

A1

(11) International Publication Number: **WO 97/48728**

(43) Internati nal Publication Date: 24 December 1997 (24.12.97)

(21) International Application Number: PCT/NL97/00345

(22) International Filing Date: 19 June 1997 (19.06.97)

(30) Priority Data:
96201720.8 20 June 1996 (20.06.96) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicant *(for all designated States except US)*: KOSTER, Henk, Wilhelmus [NL/MC]; 1607 Parc Saint Roman, MC-98000 Monte Carlo (MC).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VAN LEENGOED, Leonardus, Andrianus, Maria, Govardus [NL/NL]; Oostrandpark 6, NL-8212 AN Lelystad (NL). HOEBE, Kasper, Hubertus, Nicolaas [NL/NL]; Tolsteegplantsoen 46, NL-3523 AN Utrecht (NL). HOEBE, Kasper, Hubertus, Nicolaas [NL/NL]; Karveel 10-04, NL-8231 AP Lelystad (NL).

(74) Agent: SMULDERS, Th., A., H., J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

(57) Abstract

The invention relates to IL-6 and IL-6-receptor derived peptides having IL-6 agonistic or antagonistic activity. The peptides are at least 5 amino acids long and are selected from one of the following amino acid sequences: RYILDGISALRK, STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQMQLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, EWGPRSTPSLTTKAVLLVRKFQNSPAED or PVYKVKPNPPHNLSVIN. Selected peptides and combinations of selected peptides can be used in the treatment, prevention, detection, or diagnosis of IL-6 related disease and can be used to clear blood or bloodproducts of IL-6 or IL-6 receptor molecules.

PCT

Receiving Office use

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

Applicant's or agent's file reference (if desired) (12 characters maximum) PCT 0589

Box No. I TITLE OF INVENTION

Amino acid sequences and sources of selected peptides

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

Koster, Henk W.
1607 Parc St. Roman
MC 98000 Monte Carlo
Monaco

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State (i.e. country) of nationality: NL

State (i.e. country) of residence: MC

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)

van Leengoed, Leonardus Adrianus Maria Govardus

Oostrandpark 6
8212 AN Lelystad
the Netherlands

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box is marked, do not fill in below.)

State (i.e. country) of nationality: NL

State (i.e. country) of residence: NL

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Smulders, Th.A.H.J., c.s.
c/o VEREENIGDE OCTROOIBUREAUX
Nieuwe Parklaan 97
2587 BN The Hague
the Netherlands

Telephone No. 070 - 4166711

Facsimile No. 070 - 4166799

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

Kasper, Hubertus Nicolaas Hoebe
Tolsteegplantsoen 46
3523 AN Utrecht
the Netherlands

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

Meloen, Robert Hans
Karveel 10-04
8231 AP Lelystad
the Netherlands

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: NL

State *(i.e. country)* of residence: NL

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania
- [X] AM Armenia
- [X] AT Austria
- [X] AU Australia
- [X] AZ Azerbaijan
- [X] BA Bosnia and Herzegovina
- [X] BB Barbados
- [X] BG Bulgaria
- [X] BR Brazil
- [X] BY Belarus
- [X] CA Canada
- [X] CH and LI Switzerland and Liechtenstein
- [X] CN China
- [X] CU Cuba
- [X] CZ Czech Republic
- [X] DE Germany
- [X] DK Denmark
- [X] EE Estonia
- [X] ES Spain
- [X] FI Finland
- [X] GB United Kingdom
- [X] GE Georgia
- [X] HU Hungary
- [X] IL Israel
- [X] IS Iceland
- [X] JP Japan
- [X] KE Kenya
- [X] KG Kyrgyzstan
- [X] KP Democratic People's Republic of Korea
- [X] KR Republic of Korea
- [X] KZ Kazakstan
- [X] LC Saint Lucia
- [X] LK Sri Lanka
- [X] LR Liberia
- [X] LS Lesotho
- [X] LT Lithuania
- [X] LU Luxembourg
- [X] LV Latvia
- [X] MD Republic of Moldova
- [X] MG Madagascar
- [X] MK The former Yugoslav Republic of Macedonia
- [X] MN Mongolia
- [X] MW Malawi
- [X] MX Mexico
- [X] NO Norway
- [X] NZ New Zealand
- [X] PL Poland
- [X] PT Portugal
- [X] RO Romania
- [X] RU Russian Federation
- [X] SD Sudan
- [X] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia
- [X] SK Slovakia
- [X] TJ Tajikistan
- [X] TM Turkmenistan
- [X] TR Turkey
- [X] TT Trinidad and Tobago
- [X] UA Ukraine
- [X] UG Uganda
- [X] US United States of America
- [X] UZ Uzbekistan
- [X] VN Viet Nam

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] GH Ghana
- [X] YU Yugoslavia
- [X] ZA Zimbabwe
- [X] SL Sierra Leone

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country (in which, or for which, the application was filed)	Filing Date (day/month/year)	Application No.	Office of filing (only for regional or international application)
item (1) EP	20 June 1996 20. 06. 1996	96201720.8	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen: the two-letter code may be used):* ISA / _____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office):	Date *(day/month/year)*:	Number:
EP	7 March 1997	96201720.8

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	4	sheets
2. description	16	sheets
3. claims	2	sheets
4. abstract	1	sheets
5. drawings	5	sheets
Total	28	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

J. Renes

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00345

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/54 C07K14/715 C07K16/24 C07K16/28 C07K16/42
A61K38/17 A61K38/20 A61K39/395 G01N33/68 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI D1 Week 9607 Derwent Publications Ltd., London, GB; AN 96-065477 XP002027134 & JP 07 324 098 A (DAICEL CHEM. IND. LTD. ET AL.) , 12 December 1995 see abstract ---	1,3,6, 14-16
X	DATABASE WPI Week 9607 Derwent Publications Ltd., London, GB; AN 96-065476 XP002027135 & JP 07 324 097 A (DAICEL CHEM. IND. LTD. ET AL.) , 12 December 1995 see abstract ---	1-6, 14-16
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1997	10. 11. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Noij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00345

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Dh	DATABASE WPI Week 9035 Derwent Publications Ltd., London, GB; AN 90-266224 XP002027136 & JP 02 188 600 A (CHUGAI PHARMACEUTICAL KK) , 24 July 1990 see abstract	1,3,6, 14-18
X Dh	WO 95 04075 A (MEDVET SCIENCE PTY. LTD.) 9 February 1995 see examples 15,16 see seq. id. nos 17,36,37 see table 2	1-7, 14-16,21
X D 5	EP 0 426 857 A (KURARAY CO. LTD.) 15 May 1991 see claim 7	2,3,6,7, 16
X	US 5 210 075 A (SCHOLZ ET AL.) 11 May 1993 see examples	1-5,12, 14-16
X	C. MORTON ET AL.: "Solution structure of synthetic peptides corresponding to the C-terminal helix of interleukin-6." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 219, no. 1-2, 15 January 1994, BERLIN, GERMANY, pages 94-107, XP000645297 see page 98, left-hand column, line 50 - line 61 see page 105, right-hand column, line 20 - page 106, left-hand column, line 4	1,3,4,6, 7
A	M. KALAI ET AL.: "Participation of two Ser-Ser-Phe-Tyr repeats in interleukin-6 (IL-6) binding sites of the human IL-6 receptor." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 238, no. 3, June 1996, BERLIN, GERMANY, pages 714-723, XP000645294 see table 1	12

1

INTERNATIONAL SEARCH REPORT

Internatio pplication No.
PC1/NL 97/00345

Box I Observations where c rtain claims were found uns archable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 97/00345

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504075 A	09-02-95	AU 7341494 A	28-02-95
		CA 2168261 A	09-02-95
		EP 0715633 A	12-06-96
		JP 9501154 T	04-02-97
EP 426857 A	15-05-91	CA 2026881 A	09-08-90
		WO 9009396 A	23-08-90
		US 5171837 A	15-12-92
US 5210075 A	11-05-93	NONE	

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

SMULDERS, Th., A., H., J., c.s.
Vereenigde Octrooibureaux
Nieuwe Parklaan 97
NL-2587 BN The Hague
PAYS-BAS

Date of mailing (day/month/year) 10 November 1997 (10.11.97)	
Applicant's or agent's file reference PCT 0589	IMPORTANT NOTIFICATION
International application No. PCT/NL97/00345	International filing date (day/month/year) 19 June 1997 (19.06.97)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
KOSTER, Henk, W.	NL	MC
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
KOSTER, Henk, Wilhelmus	NL	MC
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Martine Lee
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

SMULDERS, Th., A., H., J., c.s.
Vereenigde Octro ibureaux
Nieuwe Parklaan 97
NL-2587 BN The Hague
PAYS-BAS

Date of mailing (day/month/year) 01 October 1997 (01.10.97)	
Applicant's or agent's file reference PCT 0589	**IMPORTANT NOTIFICATION**
International application No. PCT/NL97/00345	**International filing date** (day/month/year) 19 June 1997 (19.06.97)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
KASPER, Hubertus, Nicolaas, Hoebe	NL	NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
HOEBE, Kasper, Hubertus, Nicolaas	NL	NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Martine Lee
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

001699164

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

SMULDERS, Th.A.H.J.
VEREENIGDE OCTROOIBUREAUX
Nieuwe Parklaan 97
2587 BN Den Haag
PAYS-BAS

kopie aan/naar | TERMIJN 20/12-'98 Gum
13 OKT. 1998
Beantwoord voorl. def. | Bericht gezonden aan dd.
MAP PCT 0589

gedeeltelijk pos. ...

ONTVANGEN
PCT 15 OKT 1998
AMERSFOORT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	08.10.98
Applicant's or agent's file reference PCT 0589	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL97/00345	19/06/1997	20/06/1996

Applicant
KOSTER, Henk Wilhelmus et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0 Tx 523656 epmu d Fax: (+49-89) 2399-4465	Peralt Lappas. R Tel. (+49-89) 2399-8052

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
PCT 0589		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL97/00345	19/06/1997	20/06/1996
International Patent Classification (IPC) or national classification and IPC		
C07K14/54		
Applicant		
KOSTER, Henk Wilhelmus et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/01/1998	08. 10. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax. (+49-89) 2399-4465	Altmaier, A Telephone No. (+49-89) 2399-8417

Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL97/00345

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16 as originally filed

Claims, No.:

1-24 as received on 18/06/1998 with letter of 18/06/1998

Drawings, sheets:

1-5 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☒ the claims, Nos.: 1-23
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL97/00345

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	CLAIMS 2, 6-12
	No:	Claims	CLAIMS 1, 3-5, 13-24
Inventive step (IS)	Yes:	Claims	CLAIMS 2, 6-11
	No:	Claims	CLAIM 12
Industrial applicability (IA)	Yes:	Claims	CLAIMS 1-24
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET International application No. PCT/NL97/00345

1.1 The documents cited in the ISR ar labell d D1 to D8.

1.2 The claims are allowable under **Art.34(1)(b) PCT.**

AD SECTION VIII:

2. Art. 6 EPC (clarity)

2.1 The words " long " in **claims 6, 7 and 10** render the claims unclear, for obvious reasons.

2.2 The term in **claim 20** " at concentrations relatively equivalent to .... bio assay " lacks clarity (**Art. 6 PCT**). The clear limits of this - let us say - range feature are not stated in the claim and different views of the limits of the range are conceivable for different skilled persons. The claim is subject to subjective interpretation and it therefore lacks clarity.

AD SECTION V.2.:

3. Art. 33(2) and (3) over D5

3.1 The peptides of the general formula of claim 1 of **D5**, when A is a peptide fragment of the formulas of claims 2 to 13 (the formulas in claims 2 to 13 depict amino acid sequences which are - in the order of claims 2 to 13 - 21, **21, 19, 18, 20, 23, 14, 22, 23, 24, 25 and 26 amino acids long**) and when each of X and Y is single bond, are peptides of (or consisting of) 5 to 30 amino acids which bind to IL-6. They are stated in D5 (see, e.g., page 57, lines 35-to 38) to inhibit the binding of IL-6 to the IL-6 receptor and it is also stated there that the administration of the peptides to a patient with an autoimmune disease inhibits the production of autoimmune antibodies caused by binding of IL-6 to its receptor. The peptides therefore antagonize the activity of IL-6 in a patient with autoimmune

disease.

Therefore, independent claim 1 (relating to peptides consisting of **5 to 30 amino acids** and exhibiting antagonistic activity directed against IL-6) and independent **claim 3** (relating to peptides consisting **5 to 30 amino acids** and exhibiting antagonistic IL-6 activity) appear to lack novelty over D5.

3.2 The above said **19, 18 and 14 amino acid** long peptides of **D5** appear to anticipate **claim 4** (relating to peptides consisting of **5 to 20 amino acids** and exhibiting antagonistic activity directed against IL-6).

3.3 The above said 11 D5 peptides **do not anticipate claim 2.** D8 (which is, according to your last letter, prior art) shows that the IL-6 receptor consists **at least** of the alpha-chain (subunit) and of the beta-chain (subunit) (see page 714, right hand column, first full sentence). We are, however, unable to show that the above said 11 D5 peptides exhibit antagonistic activity directed against the alpha- or beta-chain of the IL-6 receptor. The peptides could bind the receptor at a different place of the receptor.

3.4 D5 also discloses the use of the above said 11 peptides for the production of antibodies, for therapeutical and diagnostic purposes and also for removing IL-6 from body fluids. Therefore, **claims 13-24** also appear to lack novelty over D5.

3.5 Note that all claims which we have not objected in above items 2.1 to 2.4 are considered novel over D5.

3.6 **Claim 12** appears not to involve an inventive step **(Art. 33(3) EPC)**. Making a mixture of at least two of the above said 11 known D5 peptides is not considered and inventive contribution to the art.

4. Art. 33(2) over D2.

4.1 The IL-6 antagonizing **decapeptide** RYILDGISAL of **D2** falls within **claim 1** (mentioning a peptide of **5-30 amino acids**), **claim 3** (mentioning a peptide of **5-30 amino acids**), **claim 4** (mentioning a peptide of **5-20 amino acids**) and

claim 5 (mentioning a peptide of **5 to 12 amino acids**). Hence, these claims appear to lack novelty.

The peptide is stated in D2 to be useful together with a carrier for treating various autoimmune diseases. Therefore, **claims 15 and 16** (relating to a pharmaceutical preparation and its use) and **24** (relating to the use of the peptide for preparing a medicament) also appear to lack novelty.

4.2 Note that all claims which we have not objected in item 4.1 are considered novel over D2.

5. Art. 33(2) and (3) EPC over D6

5.1 The IL-6 antagonizing peptides of the formula of claim 1 of **D6**, wherein X1 and Y1 are absent (note that X1 and Y1 are stated in the claim to be optional), wherein X2 = hydrogen and wherein Y2 = OH (these peptides consists of **8 amino acids**) anticipate **claims 1, 3, 4 and 5** which therefore appear to lack novelty.

5.2 The IL-6 antagonizing peptides of the formula of claim 7 of **D6**, wherein X1 and Y1 are absent, wherein X2 = hydrogen and wherein Y2 = OH (these peptides consists of **5 amino acids**) anticipate **claims 1, 3, 4 and 5** which therefore appear to lack novelty.

5.3 D6 also discloses the use of its peptides for the production of antibodies and for therapeutical and diagnostic purposes. Therefore, it also appears that **claims 13 to 16, 18, 19 and 22 to 24** lack novelty over **D6**.

5.4 Note that all claims which we have not objected in above items 5.1 to 5.3 are considered novel over D5.

5.5 **Claim 12** does not lack novelty over **D6**. But, making a mixture containing at least two of the known peptides is not considered an inventive contribution to the art. Claim 12 appears not to involve an inventive step (**Art. 33(3) EPC**).

6. The remaining documents **D1, D3, D4, D7 and D8** appear not to anticipate/

render obvious more of the claims than D2, D5 and D6 as discussed hereinbefore do.

VEREENIGDE OCTROOIBUREAUX
'S-GRAVENHAGE (HOLLAND)

Int.pat.appln. PCT/NL97/00345
our letter of 18-06-1998

Ren/PCT 0589

CLAIMS

1 A peptide of 5-30 amino acids which peptide exhibits antagonistic activity directed against IL-6.

2 A peptide of 5-30 amino acids which peptide exhibits antagonistic activity directed against the α or β-chain of the IL-6 receptor.

3 A peptide of 5-30 amino acids which peptide exhibits antagonistic or agonistic IL-6 activity.

4 A peptide according to claim 1, 2 or 3 of 5-20 amino acids.

5 A peptide according to claim 4 of 5-12 amino acids.

6 A peptide according to claim 1, 2, 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNE-GKKMRCEWDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKFNPPHNLSVIN.

7 A peptide according to claim 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTT-KAVLLVRKFQNSPAED

8 A peptide composition, wherein at least two peptides according to any of claims 1-7 are chemically linked directly or via spacer molecules.

9 A peptide composition according to claim 8 wherein at least two peptides are linked with lysine.

10 A peptide composition wherein at least two peptides according to claim 1, 2, 3, 4, or 5 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

11 A peptide composition according to claim 8, 9 or 10 wherein at least four peptides are linked with branching oligolysines.

12 A mixture comprising peptides and/or peptide compositions according to any of claims 1-11.

13 Antibody specifically directed against a peptide or a peptide composition according to any of claims 1-11.

14 Anti-idiotypic antibody raised against an antibody according to claim 13.

15 A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to any of the above claims together with at least one suitable excipient for administration.

16 Use of a pharmaceutical preparation according to claim 15 in the treatment or prevention of an IL-6 related disease.

17 Use of a peptide, peptide composition or antibody according to anyone of claims 1-13 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

18 A diagnostic assay comprising a peptide or a peptide composition or an antibody according to anyone of claims 1-13.

19 Use of a diagnostic assay according to claim 18 to detect or diagnose IL-6 related disease in man or animals.

20 Use of a peptide according to claim 7 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 µg/ml when tested in vitro in a B9 cell bio assay.

21 Use of a peptide according to claim 20 in cell-culture.

22 A pharmaceutical preparation comprising a peptide according to claim 7 together with at least one suitable excipient for administration.

23 Use of a pharmaceutical preparation according to claim 22 for topical or intra-mammary application.

24 Use of a peptide, or peptide composition according to anyone of claims 1-12 for the manufacture of a medicament for topical or intra-mammary application.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/54, 14/715, 16/24, 16/28, 16/42, A61K 38/17, 38/20, 39/395, G01N 33/68, 33/577

A1

(11) International Publication Number: **WO 97/48728**

(43) International Publication Date: 24 December 1997 (24.12.97)

(21) International Application Number: PCT/NL97/00345

(22) International Filing Date: 19 June 1997 (19.06.97)

(30) Priority Data:
96201720.8 20 June 1996 (20.06.96) EP
(34) Countries for which the regional or international application was filed: AT et al.

(71) Applicant *(for all designated States except US)*: KOSTER, Henk, Wilhelmus [NL/MC]; 1607 Parc Saint Roman, MC-98000 Monte Carlo (MC).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VAN LEENGOED, Leonardus, Andrianus, Maria, Govardus [NL/NL]; Oostrandpark 6, NL-8212 AN Lelystad (NL). HOEBE, Kasper, Hubertus, Nicolaas [NL/NL]; Tolsteegplantsoen 46, NL-3523 AN Utrecht (NL). HOEBE, Kasper, Hubertus, Nicolaas [NL/NL]; Karveel 10-04, NL-8231 AP Lelystad (NL).

(74) Agent: SMULDERS, Th., A., H., J.; Vereenigde Octrooibureaux, Nieuwe Parklaan 97, NL-2587 BN The Hague (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

(57) Abstract

The invention relates to IL-6 and IL-6-receptor derived peptides having IL-6 agonistic or antagonistic activity. The peptides are at least 5 amino acids long and are selected from one of the following amino acid sequences: RYILDGISALRK, STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQMQLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, EWGPRSTPSLTTKAVLLVRKFQNSPAED or PVYKVKPNPPHNLSVIN. Selected peptides and combinations of selected peptides can be used in the treatment, prevention, detection, or diagnosis of IL-6 related disease and can be used to clear blood or bloodproducts of IL-6 or IL-6 receptor molecules.

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BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
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IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

The invention relates to the field of cytokines. Cytokines are substances that are produced by cells of the immune system and are involved in regulation of humoral and cellular immune reactions and inflammatory responses. Many cytokines are known, and all exert influence on various reactions in the body in a complicated fashion. To illustrate their interdependency and the intricate web of relationships that exist between cytokines, one often speaks about the "cytokine network".

Interleukine 6 (IL-6) is a cytokine which has many effects upon mammalian cells. It exerts these effects through binding to a specific cell surface receptor, that consists of a specific α-subunit of with a molecular weight of approximately 80 kD and a common ß-subunit of approximately 130 kD, also named gp130. The gp130 ß chain is also involved in signal transduction of interleukin-11 (IL-11), leukemia inhibitory factor (LIF), oncostatin M (OM), ciliairy neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1) (P.B. Sehgal, Ling Wang, Ravi Rayanade et al., pp 1-14; volume 762, Annals of the New York Academy of Sciences; 1995).

IL-6 is an extremely pleiotropic cytokine, and its activities include: induction of Ig production by B cells, stimulation of B and T cell growth, differentiation of T cells and macrophages, induction of acute phase protein production by hepatocytes, multilineage hematopoesis, osteoclast formation, maturation of megakaryocytes, and platelet production. IL-6 also effects the central nervous system: IL-6 is an endogenous pyrogen and can induce ACTH production by the pituitary, finally resulting in increased glucocorticoid levels in the circulation. IL-6 exerts its activity by triggering a transmembrane receptor that is present on all target

cells. Specific steps in the IL-6 signaling cascade are the binding to the low affinity α-chain (CD126). The complex of IL-6 and α-chain binds with the high affinity signal transducing ß-chain (GP130, CD130).

In healthy individuals no or only very low levels of IL-6 (<10 pg/ml) are detectable in the circulation. IL-6 levels are increased in various diseases, and it is postulated that these increased levels play a causative role in the pathogenesis of these diseases. Examples of diseases where increased levels of IL-6 are found are multiple myeleoma, AIDS lymphoma, polyclonal B cell activation as observed in AIDS, rheumatoid arthritis, cardiac myxoma and Castleman's disease, mesangial proliferative glomerulonephritis, psoriasis, cancer-associated cachexia, postmenopausal osteoporosis, sepsis, multiple system organ failure, alcohol cirrhosis, and diseases of the central nervous system like Alzheimer, among others. Evidence for the causative role of IL-6 in the pathogenesis of some of the above mentioned diseases has come from from phase I/II clinical trials with IL-6 neutralizing monoclonal antibodies. Treatment with anti-IL-6 monoclonal antibodies reversed fever, acute phase proteins, night sweats, bone destruction, and cachexia. Treatment of a patient with Castleman disease with anti-IL-6 monoclonal antibodies reduced acute phase protein levels, fever, anemia, thrombocytosis, and hypergamma-globulinemia. Improvement of patients was also observed in patients with rheumatoid arthritis. Apparently, reduction of IL-6 activity in these patients resulted in improvement of the clinical signs of their disease.

This approach for treating disease by antagonizing IL-6 activity makes use of monoclonal antibodies directed to IL-6. However, monoclonal antibodies are usually not of human origin and repeated administration of non-human monoclonal antibodie generally leads to immune responses against the constant part of the antibodies, since this is foreign to the body of the patient. This

immunereaction to the monoclonal antibodies used in the treatment is, first of all, counterproductive to the therapeutic treatment itself. The monoclonal antibodies used will be rendered ineffective by the reaction with the antibodies produced by the immune system. Secondly, repeated administration of non-human monoclonal antibodies may elicit such severe immune reactions that they will be detrimental to the patient. Methods for producing less antigenic antibody fragments and methods for humanizing antibodies have been proposed, but, if feasible at all, these methods are not very economical and will their own give rise to problems regarding to half-life and bio-availability. Consequently, using anti-IL-6 monoclonal antibodies in the treatment of IL-6 related disease is considered not to be feasible.

Inhibitors or antagonists based upon mutagenesis of IL-6 have also been proposed, such as IL-6.Q160E /T163P (Brakenhoff, J., de Hon,F., Fontaine, V., et al; J.Biol.Chem.; 269:86-93 (1994)), and IL-6.Q159E/T162P (Ehlers, M., de Hon, D., Klaasse Bos, H, et al., J. Biol. Chem.; 270:8158-8163 (1995)). It has been shown with these mutant proteins that receptor binding of IL-6 and signal transduction of IL-6 can be separated in vitro. However, such mutant proteins are also foreign to the body of the patient to be treated and will also elicit an unwanted and unfavourable immune response that generally is detrimental to the treatment. Furthermore, such mutant proteins may only be partly effective, in that, although they may effectively block or inhibit specific IL-6 activities, at the same time they may exert other effects on the cytokine network with additional, still remaining, reactive sites present on these proteins. Therapeutic treatment with such reagents would then elicit other, yet unpredictable, side effects. A great disatvantage of earlier reported mutant IL-6 and IL-6 receptor antagonists is that these molecules, instead of inhibiting IL-6 in vivo, act as carrier and increase the

half-life time and result in an increase of IL-6 activity in vivo. Moreover, these mutant IL-6 and IL-6 receptor antagonists have a low affinity to their target molecules and will likely act as an immunogen. In addition, antibodies raised to IL-6 stabilize IL-6 and result in an increased IL-6 production. Accumulation of circulating IL-6 as stable IL-6-anti-IL-6 complexes as a result of treatment with these antibodies to IL-6, will occur as no renal filtration can be expected. Repeated use of nonhumanized IL-6 antibodies to human patients will most likely induce antibody production to these antibodies, and result in formation of immune-complexes (Heremans,H., Dillen,C., et al J.Immunol. 22,2395-2401).

The present invention provides a solution to the above illustrated problems without hampering the possibility of therapeutic treatment of IL-6 related disease. The above methods to inhibit IL-6 activity by antibodies or mutants, differ greatly from the invention as described here: peptides that antagonize or agonize IL-6 at the binding site to the receptor in three ways: at the IL-6 part, at the α-receptor part, and at the gp130-receptor part. These antagonists and agonists and combinations of these antagonists and/or agonists as multimeric peptides or as single peptides with defined pharmacokinetic characteristics gives a powerful tool to manage IL-6 bioactivity. With the solution provided by the present invention, immune responses to the treatment do not occur. Further, the occurence of unpredictable side effects is greatly minimized.

The invention provides synthetic peptides that interact with the receptor site of IL-6 or with IL-6 receptors (α and ß) present at target cells.

The invention further provides synthetic peptides that, when combined, interact with the receptor site of IL-6 as well as with IL-6 receptors (α and ß) present at target cells. A mixture of these peptides is particulary valuable as the pharmacological properties of the

peptides can be adjusted to obtain a maximal desired effect. Moreover, half-life time can be prolonged by inserting unnatural amino-acids into the synthetic peptides. The antagonizing or agonizing activity of the peptides is increased by producing di- or multi-meric peptides directed to one or more receptor sites. Such di- or multimeric peptides can for instance be made by linking the peptides via one or more amino-acids such as lysine (Tam, PNAS 1988, 85: 5409-5413). The distribution of the peptides into target organs can be optimized by adjusting the hydrophilic or lipophylic nature of peptides or by binding of these peptides onto peptides that interact with specific organ markers. Finally, the peptides provided can be bound onto the solid phase of membranes or filters that are connected into an extra-corporal blood circulation circuit of the patient. A more efficient clearance of IL-6 and/or soluble IL-6 receptors can in that way be achieved.

Such synthetic peptides can be derived from (A) IL-6, or derived from (B) the receptor α-chain of IL-6 (IL-6Rα, CD126), or from (C) the receptor ß-chain of IL-6 (IL-6Rß, GP130, CD130) and exhibit antagonistic and agonistic activity against the various components and steps of the IL-6 signaling cascade. The peptides were found by testing sets of overlapping amino acid sequences from the published human IL6 (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987), IL-6Ra (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988)) and IL-6Rß (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990)). These overlapping peptides, each twelve amino acids long, were tested in an assay for antagonistic or agonistic IL-6 activity.

The peptides provided by the invention all exhibit antagonistic or agonistic IL-6 activity against the IL-6 signaling cascade as measured in an IL-6 assay. The

peptides of the present invention are too small to generate immune responses. Further, they are too short to contain additional reactive sites, so that the antagonistic and, in addition, the agonistic peptides can advantageously be used to treat patients to counteract and adjust elevated IL-6 levels. The amino acids in all antagonistic or agonistic peptides described below are identified by the one letter code, in which the N-terminal (head) amino acid is listed first (on the left) and the C-terminal (tail) amino acid is listed last (on the right).

A. The antagonistic peptides derived from IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 3 regions that were identified as RYILDGISALRK, STKVLIQFLQKKAKNL, and I-LRSFKEFLQSSLRALRQM.

B. The antagonistic peptides derived from the receptor α-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 3 regions that were identified as QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, and MCVASSVGSKFSKTQTFQGC. The agonistic peptides derived from the receptor α-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following region that was identified as EWGPRSTPSLTTKAVLLVRKFQNSPAED.

C. The antagonistic peptides derived from the receptor ß-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 4 regions that were identified as PEKPKNLSCIVNEGKKMRCE-WDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, and PVYKVKPNPPHNLSVIN.

Relatively short peptides (as short as a string of 5 amino acids) that are selected from any of the above peptides, or peptides of no more than 30 amino acids long which show antagonistic or agonistic activity as measured in an IL-6 assay and have at least one string of at least 5 amino acids in common with the peptides from groups A,

B or C, are also peptides of the present invention. The peptides according to the invention can vary in length. Also, the peptides comprising a string of at least 5 amino acids which are in common with the peptides from groups A, B, and C can be modified by replacing one or a few amino acids in said string by other amino acids. Such amino acids can be selected from any of the naturally occuring amino acids, but also amino acids that normally do not occur in nature can be used as replacement amino acid. The choice of the replacing amino acid can for example be guided by comparing IL-6 or IL-6 receptor sequences from other species than humans or by selecting amino acids that lead not to extreme functional or conformational changes of the selected peptide, but also other selection methods can be used. More in particular, the present invention relates in a first aspect to a peptide containing at least 5 amino acids and at most 30 amino acids that exhibits antagonistic activity directed against IL-6 and/or against the α-chain of the IL-6 receptor and/or against the ß-chain of the IL-6 receptor.

Also, the present invention relates in another aspect to a peptide containing at least 5 amino acids and at most 30 amino acids that can exhibit antagonistic or agonistic IL-6 activity, depending on the concentration in which it is used. An example of such peptides are peptides selected with as basis with the amino acid sequence EWGPRSTPSLTTKAVLLVRKFQNSPAED as found in the α-chain of the IL-6 receptor. Surprisingly, peptides selected on the basis of the aforementioned sequence expressed antagonistic IL-6 activity at high concentrations whereas at low concentrations a marked agonistic activity was found. Agonistic activity was observed in the <u>in vitro</u> bioassay in a concentration range from 7.5 to 120 μg/ml peptide. At a concentration of ≥120 μg/ml these peptides had an antagonistic effect upon the biological activity of IL-6 in the bioassay. The agonistic peptides can be used <u>in vivo</u> in concentrations

that are relatively equivalent but not necessarily the same as when used in vitro.

Furthermore, the invention provides combinations of peptides, either provided as a simple mixture of several, possibly modified, peptides selected from groups A, B or C, or, provided as, possibly modified, peptides selected from groups A, B, or C that are linked, with direct chemical bonds or using spacer molecules, head to tail, or head to head, or tail to tail, or via side chains of the amino acids present in the selected peptides. Examples of such combinations of peptides are for example using the peptides SLTTKAV and ILRSFKEFLQSS, or WVEAENALGKVTSDH and RYILD, or KAVLLVRK and KAVLLVRK, but many other combinations of two or more peptides can be selected from the peptides listed in groups A, B or C. Such combinations of peptides, be it simple mixtures or bound peptides, can advantageously be used to counteract the events occuring in the IL-6 signaling cascade, such as disrupting the binding of IL-6 to the α-chain by simultanous competing at both the IL-6 and the α-chain binding site, or simultanous competing at the binding sites of the IL-6/α-chain complex and the ß-chain.

The peptides of the invention can suitably be used in a medicinal or pharmaceutical preparation for therapeutic or prophylactic purposes. Further, they can be used in protocols to remove circulating IL-6 from the blood of diseased patients via dialysis methods in which the peptides are bound to a solid phase. Passing blood or blood filtrates along the thus bound peptides will result in clearance of IL-6 that will bind to the peptide at the solid phase. Also the peptides according to the invention may be added to blood or blood filtrates and (ir)-reversibly bind to IL-6 or IL-6 receptor molecules and thus render these inactive before they re-enter the body. Also, the peptides can be used in diagnostic tests, i.e. in direct binding or competition based enzyme-linked immunosorbent assays to measure IL-6 levels.

IL-6 agonistic peptides can completely or partially replace IL-6 that is added to cell-cultures, for example IL-6 is used to grow or culture IL-6 dependant cells, like B-cell hybridomas to which IL-6 as growth factor is often added, bot also cell-cultures in general will benefit from the addition of agonistic IL-6 peptides. The IL-6 agonistic peptides administered to humans or animals can be used to enhance the immune response of an host exposed to a specific immunogenic substance. The IL-6 agonistic peptides can be administered to humans or animals to increase the responsiveness of the immune system of the host. A specific use is in pharmaceutical preparations for topical or intramammary application. When these agonistic peptides are combined with IL-6 antagonists as described, excess of IL-6 can be inhibited without loss of basal IL-6 signal transduction.

Antibodies specifically directed against the peptides, and their corresponding anti-idiotypic antibodies, are part of the invention. Such antibodies can for example be administered to patients treated earlier with the peptides, to counteract the effect of the peptides on the patient. Such antibodies can be used in the above described dialysis protocols and diagnostic tests.

Synthesis of the peptides may be acomplished according to the available methods in the art. The synthesis of the exemplified peptides was done according to Valerio et al. (Int. J. Peptide Res., 42:1-9 (1993) and/or Valerio et al. (Int. J. Peptide Res., 44:148-165 (1994)). Methods for large scale production of syntethic peptides and the purification thereof are well known in the art. The invention is illustrated in the following experimental part.

Experimental part

1. Peptide synthesis.

The peptides of the examples which were intended for identifying active centers in the IL-6 and IL-6 receptor molecules were synthesized using a method according to Valerio et al. (Int. J. Peptide Res., 42:1-9 (1993) and/or Valerio et al. (Int. J. Peptide Res., 44:148-165 (1994)). Multimeric peptides (four branched) were synthetized by the solid-phase method and using of a dispersed system with branching oligolysines as a scaffolding for incorporation of the synthetized antagonistic peptides (Tam, J.P.; Proc. Natl. Acad. Sci. USA, 85:5409-5413 (1988).

2. Proliferation assay to determine antagonistic IL-6 activity.

A set of overlapping peptides, each twelve amino acids long (each consecutive peptide shifts one amino acid, so consecutive peptides have 11 amino acids in common), derived from human IL-6 sequence (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987), were incubated with cells (B9) at 37°c. After one hour, recombinant human IL-6 (CLB, Amsterdam, The Netherlands) was added at 3 different concentrations (2.5 U/ml, 5 U/ml and 10 U/ml).

A set of overlapping peptides, each twelve amino acids long (each consecutive peptide shifts one amino acid, so consecutive peptides have 11 amino acids in common), derived from human IL6Ra (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988)) or gp130 (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990)), were incubated with 3 different concentrations IL-6 (2.5 U/ml, 5 U/ml, 10 U/ml) diluted in DMEM supplemented with HT for one hour at 37°c. Then the residual IL-6 activity was determined in a biological

assay by measuring the IL-6 dependant proliferative growth of B9 mouse hybridoma cells (Helle, M., Boeije, L., Aarden, L.A.; Eur. J. Immunol. 18:1535-1540 (1988)). Briefly, B9 mouse hybridoma cells were collected during their logarithmic growth phase in IL-6 free media and suspended at a concentration of $1*10^5$ cells/ml in DMEM+HT medium containing 5% FCS. Fifty µl of each IL-6 dilution was combined with each of the synthetized peptides representing IL-6 sequences and incubated for 1 hour at 37°C. This mixture was added in duplicate to 50 µl of the B9 cell suspension in flat-bottommed 96-well tissue culture plates (Greiner) and incubated at 37°c and 5% CO_2 for 72 h. IL-6 activity was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After addition of 25 µl of MTT (5mg/ml dissolved in PBS) to each well and further incubation at 37°c for 4 h, 100 µl of lysis buffer (20% w/v SDS in 50 % dimethyl formamide) was added. Thereafter, incubation was continued over night at 37°c and the next morning absorbance was read at 578 nm.

To determine the agonistic or antagonistic activity to IL-6 of the peptides synthetized from the sequences of the IL-6 receptor a or ß, various concentrations of each of these peptides was combined with 50 µl of the B9 cell suspension ($1*10^5$ cells/ml in DMEM+HT medium containing 5% FCS). This suspension was incubated for 1 hour at 37°C, and combined with each of the dilutions of IL-6 into flat-bottommed 96-well tissue culture plates (Greiner). Plates were incubated at 37°C for 72 h. IL-6 activity was assessed as described above.

Samples without synthetized peptides or with a sham peptide but with IL-6 were used as positive control, whereas samples that contained neither IL-6 nor synthetized peptides were used as negative control.

Inhibition or enhancement of IL-6 activity was determined by calculating the ratio absorbance of test sample and

absorbance positive control both corrected for negative control absorban ce.

3. Toxicity testing of peptides

Three separate test were performed to determine whether the synthetized peptides excert toxic effect in vitro upon erythrocytes (A), or polymorphonuclear cells (B), or hepatocytes (C).

A. Sheep red blood cells (SRBC) were washed five times in PBS. A 1% (v/v) suspension of erythrocytes was prepared in veronal-buffered saline that contained gelatin (GVS: 0.032% gelatin in 3.9 mM barbitone sodium, 1 mM $MgSO_4$, 0.38 mM $CaCl_2$, and 145.6 mM NaCl). Twofold dilutions of the synthetized peptides (50 μl) were made in U-shaped microtiter plates (Greiner Labortechnik) and 50 μl of the SRBC suspension were added to each well. Plates were sealed, mixed and incubated for 2 hours at 37°C. Thereafter, plates were examined for hemolysis. None of the synthetized peptides showed hemolysis.

B. Porcine polymorphonuclear cells (PMN) were isolated from pig blood (Cruijsen, T.L.M., Van Leengoed, L.A.M.G. et al.; Infect. Immun. 60:4867-4871 (1992)). Twofold dilutions of the synthetized peptides (50 μl) were made in flat-bottomed microtiter plates (Greiner Labortechnik) and 50 μl of the PMN suspension ($2*10^6$ cells/ml) were added to each well. Plates were sealed, gently mixed and incubated for 6 hours at 37° and 5% CO_2. Thereafter, plates were examined for cytotoxicity by nigrosine dye exclusion. Non of the synthetized peptides was toxic for PMN.

C. Porcine hepatocytes were isolated from liver of pigs based on Seglens' method (Seglen, P.O.; Methods Cell Biol 13:29-83 (1976)) and adapted according to Monshouwer M., et al. (Toxicol. Applied Pharmacol.in press). Hepatocytes were suspended in Williams' medium E to a concentration of 10^6 cells/ml. From this suspension 1.5 ml was put into each well of 12-well tissue culture plates (Costar) and

incubated for 12 h at 37°C. Adherent hepatocytes were examined for their viability and nonadherent hepatocytes were discarded. Each synthetized peptide was mixed with Williams' medium E (at dilutions of 1:50 and 1:100) and added to wells with adherent hepatocytes. After another 24 h incubation at 37°C viability was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After addition of 1.5 of MTT (1-mg/ml dissolved in Williams' medium E) to each well and further incubation at 37°c for 30 min, 1 ml of lysis buffer (0.8 M HCL in isopropanol) was added. Thereafter, plates were mixed for 10 min and absorbance was read at 560 nm. None of the synthetized peptides proved to affect the viability of the hepatocytes.

4. Effect of IL-6 antagonistic peptides upon IL-6 induced acute phase reaction and downregulation of hepatic biotransformation activities.

Porcine hepatocytes were isolated from liver of pigs based on Seglens' method (Seglen, P.O.; Methods Cell Biol 13:29-83 (1976)) and adapted according to Monshouwer M., et al. (Toxicol. Applied Pharmacol.in press). Hepatocytes were suspended in Williams' medium E to a concentration of 10^6 cells/ml. From this suspension 1 ml was put into each well of 12-well tissue culture plates (Costar) and incubated for 12 h at 37°C. Adherent hepatocytes were examined for their viability and nonadherent hepatocytes were discarded. Each synthetized peptide was mixed (at dilutions of 1:50 and 1:100) with Williams' medium E containing IL-6 (1000 U/ml) and added to wells with adherent hepatocytes. Also a negative (containing no IL-6 and without synthetized peptides in the medium) and positive control (containing 1000 U/ml IL-6 in the medium) were prepared and tested. After an incubation period of 24 hours, the medium was removed and for each well CYP450 dependent enzyme activity of intact monolayers of hepatocytes was determined.

CYP450 enzym assay. CYP450 dependent enzym activity, using testosterone (250μM) as substrate, was determined as previously described by Van 't Klooster et al.(Bioch. Pharmacol.46;1781-1790 (1993)). Briefly, testosteron was mixed with Williams' medium E without fetal calf serum and added to the wells with hepatocytes. After 30 min incubation at 37°C and 5%CO_2, hydroxylated testosteron metabolites in the medium were quantified by HPLC.

HPLC analysis. Aliquots of 1 ml of medium was mixed with 100 μl of a solution of 11ß-testosteron (12,5 μg/ml) in methanol as internal standard and extracted with 5 ml dichlormethane. The organic phase was transferred to clean tubes and evaporated to dryness at roomtemperature under a stream of nitrogen. The residues were dissolved in 130 μl 50% methanol and 20 μl of these solutions were injected for HPLC analysis. The stationary phase consisted of a C18 glasscolumn (20 cm, 3μm particle size, Chrompack, Middelburg, the Netherlands). The mobile phase consisted of buffer A (12% methanol, 75% milli Q water) and buffer B (64% methanol, 6% acetonitril, 30% milli Q water). With these buffers an elution gradient was generated; 10-58% B from 0-45 minutes; 58-59% from 45-50 minutes; 59-10% from 50-53 minutes, with a flow rate of 0,8 ml/min. Metabolites were detected spectrofotometrically at 254 nm. Inhibition of IL-6 dependant downregulation of cytochrome P450 was determined by comparing the relative concentration of hydroxylated testosteron metabolites in medium from adherent hepatocytes incubated with synthetized peptides and IL-6, and the relative concentration of hydroxylated testosteron metabolites in medium from positive and negative control hepatocyte monolayers.

5. Results

Peptides derived from hIL-6, hgp130 (the ß-chain of the IL-6 receptor) and hIL6Ra (the α-chain of the IL-6 receptor) were analysed for antagonistic IL-6 activity.

For hIL-6 peptides, 3 regions were identified which inhibited IL-6 activity in an IL-6 assay (fig. 2). Peptide 31, 119-123 and 167-174 represent the identified regions (RYILDGISALRK, resp. STKVLIQFLQKKAKNL, resp. ILRSFKEFLQSSLRALRQM).

For hIL6Ra, also 3 regions were identified which inhibited IL-6 activity in an IL-6 asay (fig. 3). Peptide 6-9, 24-33 and 80-89 represent the identified regions (QLSCFRKSPLSNVVC, resp. PRSTPSLTTKAVLLVRKFQNS, resp. MCVASSVGSKFSKTQTFQGC).

For hgp130 peptides, 4 regions were identified which inhibited IL-6 activity in an IL-6 assay (fig. 4). Peptide 2-15, 33-46, 73-76 and 92-97 represent the identified regions (PEKPKNLSCIVNEGKKMRCEWDGGR, resp. NFTLKSE-WATHKFADCKAKRDTPTS, resp. WVEAENALGKVTSDH, resp. PVYKVKPNPPHNLSVIN).

The identified peptides with anti-IL-6 activity were not lytic for erythrocytes and not toxic for polymorpho-nuclear cells and not toxic for primary hepatocyte culture cells.

Peptides derived from hIL6Ra (the α-chain of the IL-6 receptor) were analysed for agonistic IL-6 activity and 1 region was identified which stimulated proliferation of B9 cells without IL-6 added to the medium and enhanced IL-6 activity in the B9 bio-assay (fig. 5). Peptide 21-37 represent the region EWGPRSTPSLTTKAVLLVRKFQNSPAED of the IL-6Ra sequence

Agonistic activity was observed in a concentration range from 7.5 to 120 μg/ml peptide. These peptides induced prolife rative growth of the IL-6 dependant cell line B9, and when combined with IL-6 enhanced proliferation of the B9 cell line was examined, and thus the biological activity of IL-6 was enhanced. At a concentration of ≥120 μg/ml these agonistic peptides had an antagonistic effect upon the biological activity of IL-6.

The identified peptides with agonistic IL-6 activity were not lytic for erythrocytes and not toxic for polymorphonuclear cells and not toxic for primary hepatocyte culture cells.

Synthetized peptides from the regions PVYKVKPNPP-HNLSVIN, WVEAENALGKVTSDH, and MCVASSVGSKFSKTQTFQGC inhibit IL-6 regulated downregulation of cytochrome P-450 of hepatocytes.

CLAIMS

1 A peptide containing 5-30 amino acids which peptide exhibits antagonistic activity directed against IL-6.

2 A peptide containing 5-30 amino acids which peptide exhibits antagonistic activity directed against the α and/or β-chain of the IL-6 receptor.

3 A peptide containing 5-30 amino acids which peptide exhibits antagonistic and/or agonistic IL-6 activity.

4 A peptide according to claim 1, 2 or 3 containing 5-20 amino acids.

5 A peptide according to claim 4 containing 5-12 amino acids.

6 A peptide according to claim 1, 2, 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: RYILDGISALRK, STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKS-EWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

7 A peptide according to claim 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLVRKFQNSPAED

8 A peptide composition, wherein at least two peptides according to any of claims 1-7 are chemically linked directly or via spacer molecules.

9 A peptide composition according to claim 8 wherein at least two peptides are linked with lysine.

10 A peptide composition according to claim 8 wherein at least four peptides are linked with branching oligolysines.

11 A mixture comprising peptides and/or peptide compositions according to any of claims 1-10.

12 Antibody specifically directed against a peptide or a peptide composition according to any of claims 1-10.

13 Anti-idiotypic antibody raised against an antibody according to claim 12.

14 A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to any of the above claims together with at least one suitable excipient for administration.

15 Use of a pharmaceutical preparation according to claim 14 in the treatment or prevention of an IL-6 related disease.

16 Use of a peptide, peptide composition or antibody according to anyone of claims 1-12 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

17 A diagnostic assay comprising a peptide or a peptide composition or an antibody according to anyone of claims 1-12.

18 Use of a diagnostic assay according to claim 17 to detect or diagnose IL-6 related disease in man or animals.

19 Use of a peptide according to claim 7 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 μg/ml.

20 Use of a peptide according to claim 19 in cell-culture.

21 A pharmaceutical preparation comprising a peptide according to claim 7 or 19 together with at least one suitable excipient for administration.

22 Use of a pharmaceutical preparation according to claim 21 for topical or intra-mammary application.

23 Use of a peptide, or peptide composition according to anyone of claims 1-11 for the manufacture of a medicament for topical or intra-mammary application.

Fig. 1. Amino acid sequences and sources of selected peptides.

Peptides were selected from published sequences of IL-6 (A) and IL-6 a- (B) and ß-receptor (C).

A) human IL6 (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987)
Amino acid sequence:
APPVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCESSK-
EALAENNLNLPKMAEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQ-
ARAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTH-
LILIRSFKEFLQSSLRALRQM

B) the receptor a-chain of IL-6 (IL-6Ra, CD126), (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988))
Amino acid sequence:
PPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQNSPAEDFQEPCQY-
SQESQKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANITV

C) the receptor ß-chain of IL-6 (IL-6Rß, GP130, CD130) (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990))
Amino acid sequence:
PPEKPKNLSCIVNEGKKMRCEWDGGRETHLETNFTLKSEWATHKFADCKAKRDT-
PTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNLSVIN

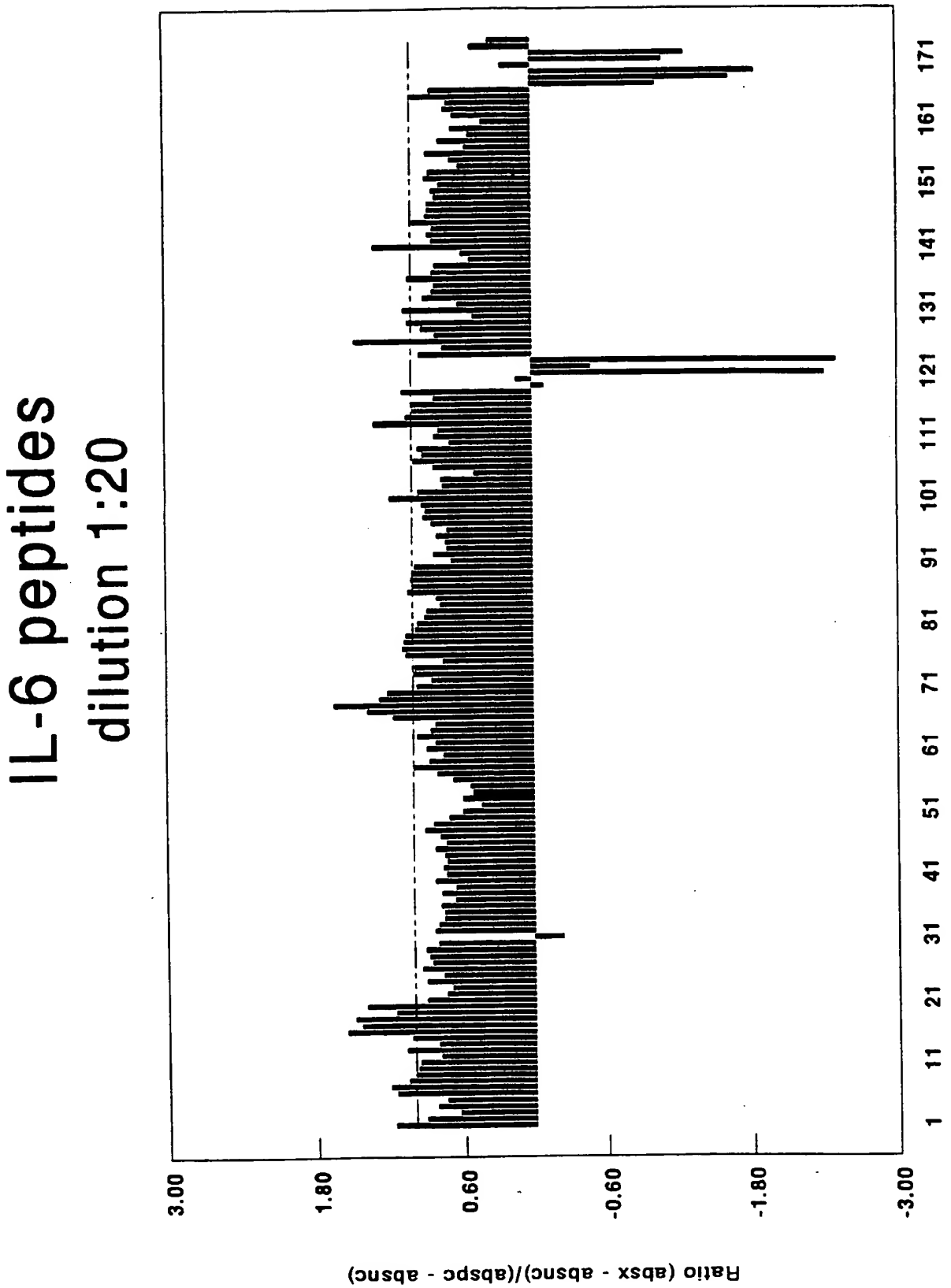

Fig 2: Screening of synthetized peptides, representing IL-6, in the B9 bio-assay

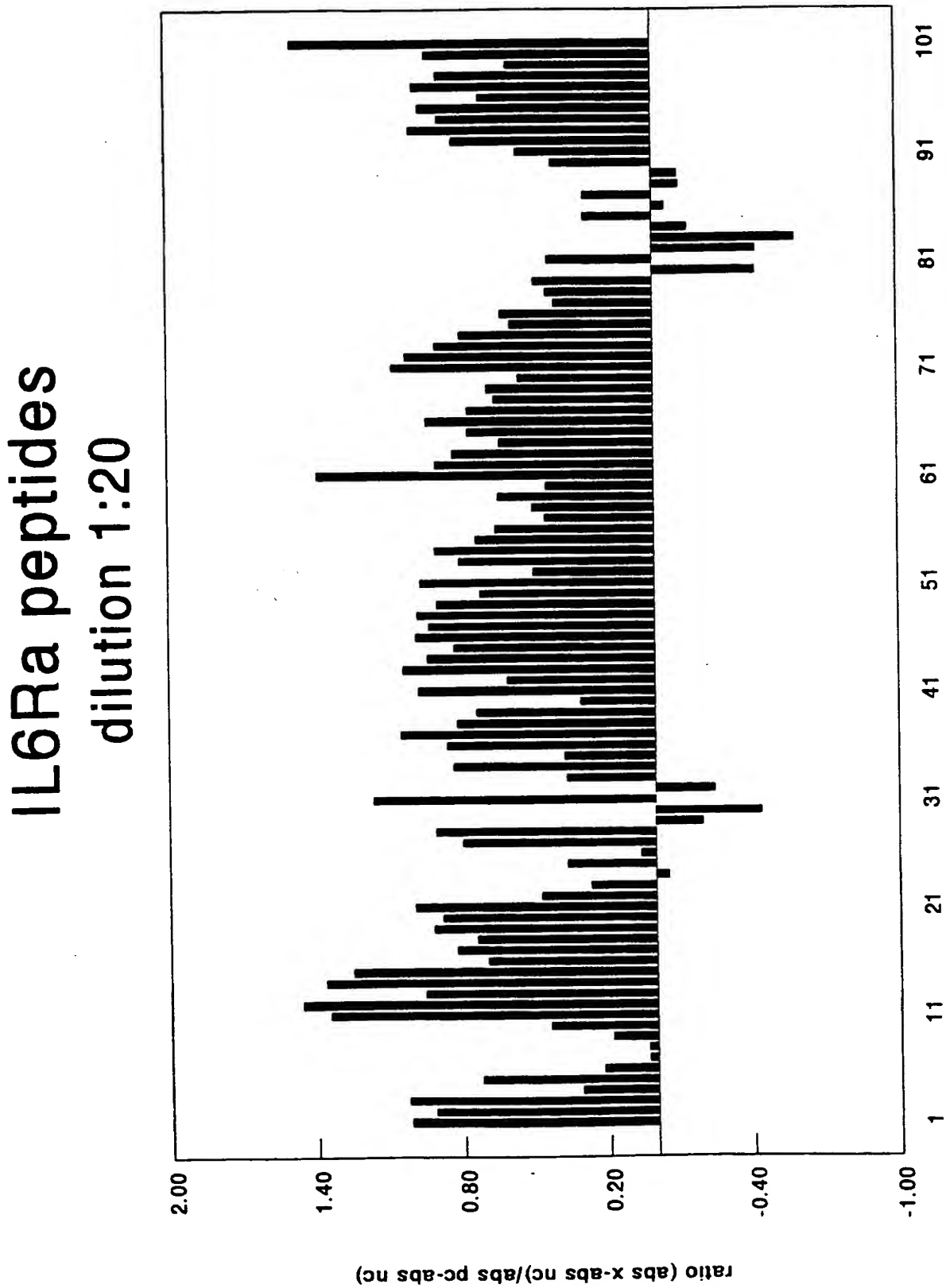

Fig 3: Scr ning of synth tiz d peptides, repr s nting IL-6Ra, in th B9 bio-assay

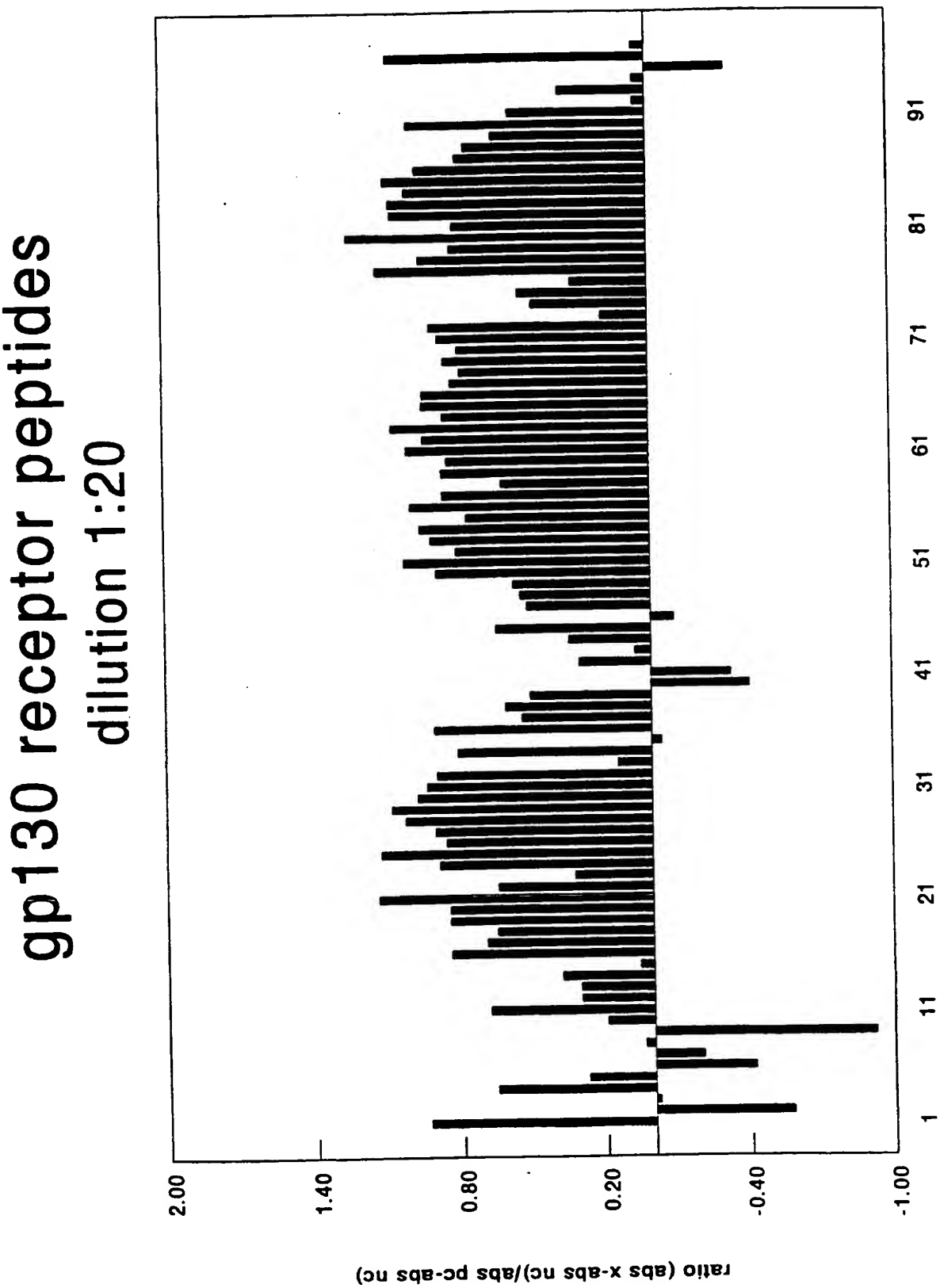

Fig 4: Scre ning of synth tiz d peptides, r presenting gp130, in the B9 bioassay

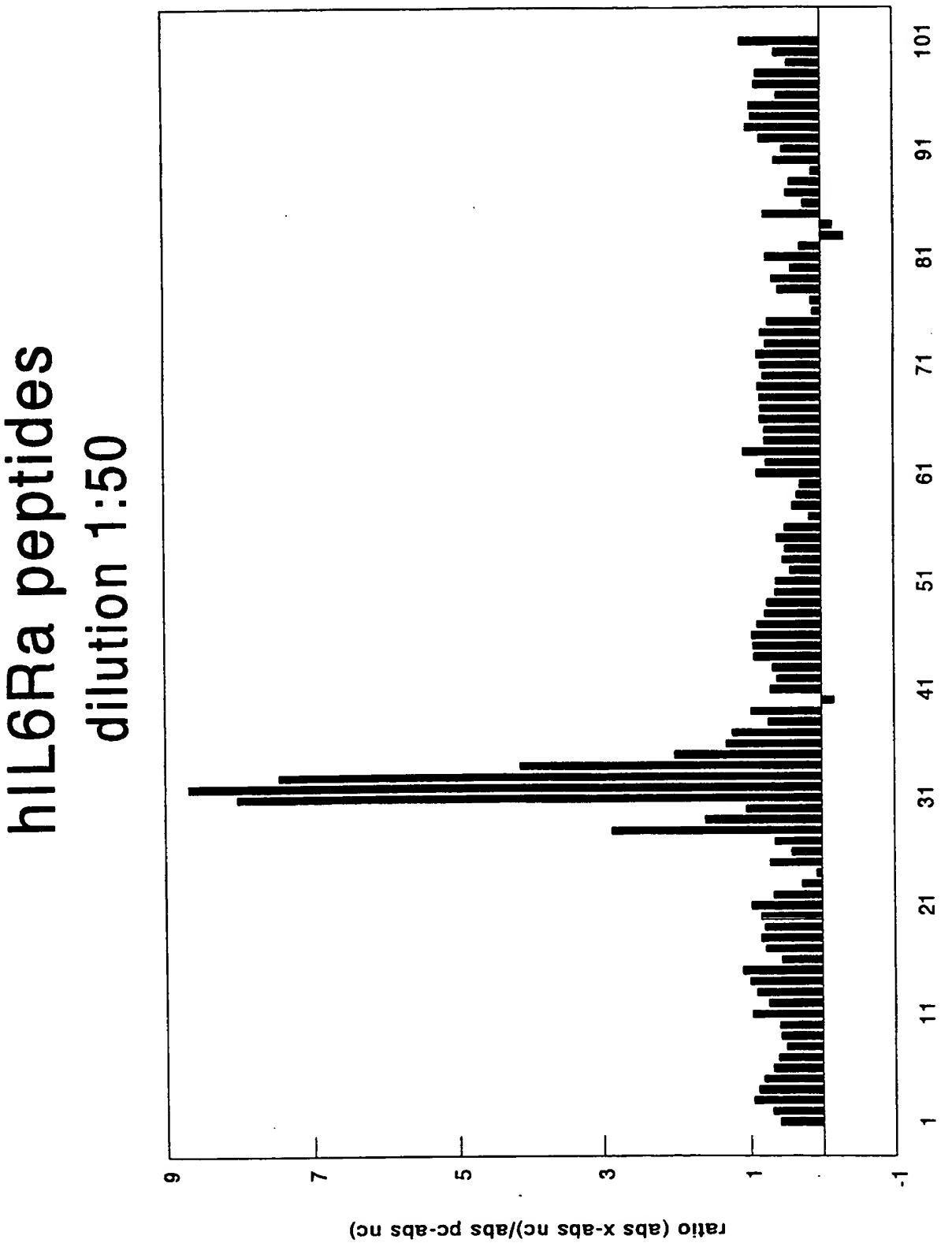

Fig 5: Screening synthetic peptides showing a region with agonistic activity (dilution 1:50)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
PCT 0589		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL97/00345	19/06/1997	20/06/1996
International Patent Classification (IPC) or national classification and IPC		
C07K14/54		
Applicant		
KOSTER, Henk Wilhelmus et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20/01/1998	05.10.98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0. Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Altmaier, A Telephone No. (+49-89) 2399-8417

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL97/00345

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-16 as originally filed

Claims, No.:

1-24 as received on 18/06/1998 with letter of 18/06/1998

Drawings, sheets:

1-5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 1-23
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	CLAIMS 2, 6-12
	No:	Claims	CLAIMS 1, 3-5, 13-24
Inventive step (IS)	Yes:	Claims	CLAIMS 2, 6-11
	No:	Claims	CLAIM 12
Industrial applicability (IA)	Yes:	Claims	CLAIMS 1-24
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1.1 **The documents cited in the ISR are labelled D1 to D8.**

1.2 The claims are allowable under **Art.34(1)(b) PCT.**

AD SECTION VIII:

2. **Art. 6 EPC (clarity)**

2.1 The words " long " in **claims 6, 7 and 10** render the claims unclear, for obvious reasons.

2.2 The term in **claim 20** " at concentrations relatively equivalent to .... bio assay " lacks clarity (**Art. 6 PCT**). The clear limits of this - let us say - range feature are not stated in the claim and different views of the limits of the range are conceivable for different skilled persons. The claim is subject to subjective interpretation and it therefore lacks clarity.

AD SECTION V.2.:

3. **Art. 33(2) and (3) over D5**

3.1 The peptides of the general formula of claim 1 of **D5**, when A is a peptide fragment of the formulas of claims 2 to 13 (the formulas in claims 2 to 13 depict amino acid sequences which are - in the order of claims 2 to 13 - 21, **21, 19, 18, 20, 23, 14, 22, 23, 24, 25 and 26 amino acids long**) and when each of X and Y is single bond, are peptides of (or consisting of) 5 to 30 amino acids which bind to IL-6. They are stated in D5 (see, e.g., page 57, lines 35-to 38) to inhibit the binding of IL-6 to the IL-6 receptor and it is also stated there that the administration of the peptides to a patient with an autoimmune disease inhibits the production of autoimmune antibodies caused by binding of IL-6 to its receptor. The peptides therefore antagonize the activity of IL-6 in a patient with autoimmune

disease.

Therefore, independent claim 1 (relating to peptides consisting of **5 to 30 amino acids** and exhibiting antagonistic activity directed against IL-6) and independent **claim 3** (relating to peptides consisting **5 to 30 amino acids** and exhibiting antagonistic IL-6 activity) appear to lack novelty over D5.

3.2 The above said **19, 18 and 14 amino acid** long peptides of **D5** appear to anticipate **claim 4** (relating to peptides consisting of **5 to 20 amino acids** and exhibiting antagonistic activity directed against IL-6).

3.3 The above said 11 D5 peptides **do not anticipate claim 2**. D8 (which is, according to your last letter, prior art) shows that the IL-6 receptor consists **at least** of the alpha-chain (subunit) and of the beta-chain (subunit) (see page 714, right hand column, first full sentence). We are, however, unable to show that the above said 11 D5 peptides exhibit antagonistic activity directed against the alpha- or beta-chain of the IL-6 receptor. The peptides could bind the receptor at a different place of the receptor.

3.4 D5 also discloses the use of the above said 11 peptides for the production of antibodies, for therapeutical and diagnostic purposes and also for removing IL-6 from body fluids. Therefore, **claims 13-24** also appear to lack novelty over D5.

3.5 Note that all claims which we have not objected in above items 2.1 to 2.4 are considered novel over D5.

3.6 **Claim 12** appears not to involve an inventive step **(Art. 33(3) EPC)**. Making a mixture of at least two of the above said 11 known D5 peptides is not considered and inventive contribution to the art.

4. Art. 33(2) over D2.

4.1 The IL-6 antagonizing **decapeptide** RYILDGISAL of **D2** falls within **claim 1** (mentioning a peptide of **5-30 amino acids**), **claim 3** (mentioning a peptide of **5-30 amino acids**), **claim 4** (mentioning a peptide of **5-20 amino acids**) and

claim 5 (mentioning a peptide of **5 to 12 amino acids**). Hence, these claims appear to lack novelty.

The peptide is stated in D2 to be useful together with a carrier for treating various autoimmune diseases. Therefore, **claims 15 and 16** (relating to a pharmaceutical preparation and its use) and **24** (relating to the use of the peptide for preparing a medicament) also appear to lack novelty.

4.2 Note that all claims which we have not objected in item 4.1 are considered novel over D2.

5. Art. 33(2) and (3) EPC over D6

5.1 The IL-6 antagonizing peptides of the formula of claim 1 of **D6**, wherein X1 and Y1 are absent (note that X1 and Y1 are stated in the claim to be optional), wherein X2 = hydrogen and wherein Y2 = OH (these peptides consists of **8 amino acids**) anticipate **claims 1, 3, 4 and 5** which therefore appear to lack novelty.

5.2 The IL-6 antagonizing peptides of the formula of claim 7 of **D6**, wherein X1 and Y1 are absent, wherein X2 = hydrogen and wherein Y2 = OH (these peptides consists of **5 amino acids**) anticipate **claims 1, 3, 4 and 5** which therefore appear to lack novelty.

5.3 D6 also discloses the use of its peptides for the production of antibodies and for therapeutical and diagnostic purposes. Therefore, it also appears that **claims 13 to 16, 18, 19 and 22 to 24** lack novelty over **D6**.

5.4 Note that all claims which we have not objected in above items 5.1 to 5.3 are considered novel over D5.

5.5 **Claim 12** does not lack novelty over **D6**. But, making a mixture containing at least two of the known peptides is not considered an inventive contribution to the art. Claim 12 appears not to involve an inventive step (**Art. 33(3) EPC**).

6. The remaining documents **D1, D3, D4, D7 and D8** appear not to anticipate/

render obvious more of the claims than D2, D5 and D6 as discussed hereinbefore do.

Replaced by Article 34 Amdt

CLAIMS

1 A peptide containing 5-30 amino acids which peptide exhibits antagonistic activity directed against IL-6.

2 A peptide containing 5-30 amino acids which peptide exhibits antagonistic activity directed against the α and/or β-chain of the IL-6 receptor.

3 A peptide containing 5-30 amino acids which peptide exhibits antagonistic and/or agonistic IL-6 activity.

4 A peptide according to claim 1, 2 or 3 containing 5-20 amino acids.

5 A peptide according to claim 4 containing 5-12 amino acids.

6 A peptide according to claim 1, 2, 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: RYILDGISALRK, STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKS-EWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

7 A peptide according to claim 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLVRKFQNSPAED

8 A peptide composition, wherein at least two peptides according to any of claims 1-7 are chemically linked directly or via spacer molecules.

9 A peptide composition according to claim 8 wherein at least two peptides are linked with lysine.

10 A peptide composition according to claim 8 wherein at least four peptides are linked with branching oligolysines.

Replaced by Article 34 Amdt

11 A mixture comprising peptides and/or peptide compositions according to any of claims 1-10.

12 Antibody specifically directed against a peptide or a peptide composition according to any of claims 1-10.

13 Anti-idiotypic antibody raised against an antibody according to claim 12.

14 A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to any of the above claims together with at least one suitable excipient for administration.

15 Use of a pharmaceutical preparation according to claim 14 in the treatment or prevention of an IL-6 related disease.

16 Use of a peptide, peptide composition or antibody according to anyone of claims 1-12 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

17 A diagnostic assay comprising a peptide or a peptide composition or an antibody according to anyone of claims 1-12.

18 Use of a diagnostic assay according to claim 17 to detect or diagnose IL-6 related disease in man or animals.

19 Use of a peptide according to claim 7 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 μg/ml.

20 Use of a peptide according to claim 19 in cell-culture.

21 A pharmaceutical preparation comprising a peptide according to claim 7 or 19 together with at least one suitable excipient for administration.

22 Use of a pharmaceutical preparation according to claim 21 for topical or intra-mammary application.

23 Use of a peptide, or peptide composition according to anyone of claims 1-11 for the manufacture of a medicament for topical or intra-mammary application.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00345

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/54 C07K14/715 C07K16/24 C07K16/28 C07K16/42
A61K38/17 A61K38/20 A61K39/395 G01N33/68 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9607 Derwent Publications Ltd., London, GB; AN 96-065477 XP002027134 & JP 07 324 098 A (DAICEL CHEM. IND. LTD. ET AL.) , 12 December 1995 see abstract ---	1,3,6, 14-16
X	DATABASE WPI Week 9607 Derwent Publications Ltd., London, GB; AN 96-065476 XP002027135 & JP 07 324 097 A (DAICEL CHEM. IND. LTD. ET AL.) , 12 December 1995 see abstract --- -/--	1-6, 14-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1997	10. 11. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Week 9035 Derwent Publications Ltd., London, GB; AN 90-266224 XP002027136 & JP 02 188 600 A (CHUGAI PHARMACEUTICAL KK) , 24 July 1990 see abstract	1,3,6, 14-18
X	WO 95 04075 A (MEDVET SCIENCE PTY. LTD.) 9 February 1995 see examples 15,16 see seq. id. nos 17,36,37 see table 2	1-7, 14-16,21
X	EP 0 426 857 A (KURARAY CO. LTD.) 15 May 1991 see claim 7	2,3,6,7, 16
X	US 5 210 075 A (SCHOLZ ET AL.) 11 May 1993 see examples	1-5,12, 14-16
X	C. MORTON ET AL.: "Solution structure of synthetic peptides corresponding to the C-terminal helix of interleukin-6." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 219, no. 1-2, 15 January 1994, BERLIN, GERMANY, pages 94-107, XP000645297 see page 98, left-hand column, line 50 - line 61 see page 105, right-hand column, line 20 - page 106, left-hand column, line 4	1,3,4,6, 7
A	M. KALAI ET AL.: "Participation of two Ser-Ser-Phe-Tyr repeats in interleukin-6 (IL-6) binding sites of the human IL-6 receptor." EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 238, no. 3, June 1996, BERLIN, GERMANY, pages 714-723, XP000645294 see table 1	12

INTERNATIONAL SEARCH REPORT

Internatic pplication No.
PCI/NL 97/00345

Box I Observations wh re certain claims were found unsear hable (Continuation of it m 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 97/00345

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504075 A	09-02-95	AU 7341494 A	28-02-95
		CA 2168261 A	09-02-95
		EP 0715633 A	12-06-96
		JP 9501154 T	04-02-97
EP 426857 A	15-05-91	CA 2026881 A	09-08-90
		WO 9009396 A	23-08-90
		US 5171837 A	15-12-92
US 5210075 A	11-05-93	NONE	

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/52, 14/505, 14/535, 14/54, 14/55, 14/475, C12N 15/19, 15/24, 15/26, 15/27, C12P 21/02, A61K 38/18, 38/19, 38/20

A1

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(43) International Publication Date: 9 February 1995 (09.02.95)

(21) International Application Number: PCT/AU94/00432

(22) International Filing Date: 28 July 1994 (28.07.94)

(30) Priority Data:

PM 0186	28 July 1993 (28.07.93)	AU
PM 4772	30 March 1994 (30.03.94)	AU

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD).

Published
With international search report.

(54) Title: HAEMOPOIETIC GROWTH FACTOR ANTAGONISTS

(57) Abstract

The present invention relates to modified and variant forms of haemopoietic growth factors (HGF) capable of acting as antagonists to the corresponding native haemopoietic growth factors and their use in ameliorating aberrant effects caused by the native molecules. A modified haemopoietic growth factor (HGF) is characterized by being in unglycosylated form and comprising a sequence of amino acids within a first α-helix wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue. The preferred HGF are granulocyte-macrophage colony-stimulating factor (GM-CSF), interleuking (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, G-CSF and erythropoietin (EPO).

HAEMOPOIETIC GROWTH FACTOR ANTAGONISTS

The present invention relates to modified and variant forms of haemopoietic growth factors capable of acting as antagonists to the corresponding native haemopoietic growth factors and their use in ameliorating aberrant effects caused by the native molecules.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is one member of a family of haemopoietic growth factors (HGFs) which have a similar predicted tertiary configuration (Parry *et al*, 1988) and whose receptors also belong to a common family (Gearing *et al*, 1989, Bazan, 1990). This family of haemopoietic growth factors includes, for example, in addition to GM-CSF, the cytokines IL-2, IL-3, IL-5, IL-6 and IL-10. A distinct subfamily comprising GM-CSF, IL-3 and IL-5 can be discerned based on structural similarities (Goodall *et al*, 1993) and on their ability to interact with a common receptor component (Lopez *et al*, 1992).

Human GM-CSF (hGM-CSF) comprises 127 amino acids and is available in recombinant form (rhGM-CSF). The hGM-CSF receptor has also been cloned and shown to comprise a binding (α) chain exhibiting low affinity binding to GM-CSF (Gearing *et al*, 1989) and a second (β) chain which does not measurably bind GM-CSF by itself but it allows the formation of a high affinity receptor when co-expressed with the α chain (Hayashide *et al*, 1990).

GM-CSF exhibits a range of activities extending over neutrophil, eosinophil and monocyte lineages. Specifically, GM-CSF stimulates the progenitors of these cells to proliferate and differentiate to become mature cells. In addition, it stimulates mature cells to greater function. The stimulation of mature cells results in greater capacity to phagocytose and kill micro-organisms, kill antibody-coated tumour cells and parasites and generate superoxide anion (O_2^-) in response to various stimuli. The purpose of this activation is presumed to enable the mature cells to become better effector cells in inflammatory reactions.

Therapeutically, the HGFs form an important group of molecules for their actual or potential properties. For example, the main indications for GM-CSF are for its effects on progenitor cells or mature cells. Using its effects on progenitor cells, GM-CSF is used in the treatment of bone marrow failure as seen in aplastic anaemia or chemotherapy or AIDS-induced marrow suppression. In the treatment of infections, the capacity to stimulate mature cells is especially relevant. The capacity of GM-CSF-activated neutrophils and eosinophils to kill tumour cells that have bound antibody is especially remarkable and could be used in tumour therapy.

However, despite the actual and potential benefits of HGFs, they can exhibit some detrimental side effects. For example, GM-CSF can exhibit toxicity due to stimulation of mature cells causing blood vessel damage or thrombosis. The eosinophilia caused by GM-CSF appears especially damaging in this regard. The molecule can also have detrimental effects by stimulating growth of leukaemia cells and tumour cells of non-haemopoietic origin and stimulating production of inflammatory mediators.

International Patent Application No. PCT/AU89/00177 and an article by Lopez *et al.* (1992) disclose amino acid variants of GM-CSF which have exhibited reduced potency. These variants were investigated further for their potential as GM-CSF antagonists. However, the variants cause classical stimulation at concentrations 100 fold greater compared to the native GM-CSF molecule. Furthermore, attempts to find antagonistic properties failed since mixing large concentrations of one of the variants with suboptimal concentrations of native GM-CSF resulted in stronger GM-CSF stimulation with no

evidence of inhibition being observed.

There is a need, therefore, to develop antagonists to HGFs and in particular GM-CSF which are capable of ameliorating the aberrant effects of the corresponding native molecules. There is also a need for such antagonists not to exhibit agonist properties in respect of the corresponding HGFs.

Accordingly, one aspect of the present invention provides a haemopoietic growth factor characterised by being in unglycosylated form and comprising a sequence of amino acids within a first α-helix wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue.

In accordance with the present invention, it is proposed that the modified HGFs defined above act as antagonists of the native form of the corresponding HGF but not other HGFs. The term "modified" is considered herein synonymous with terms such as "variant", "derivative" and "mutant".

The HGFs are preferably GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, granulocyte colony-stimulating factor (G-CSF) and erythropoietin (EPO) modified in accordance with the present invention. Most preferably, the HGF is GM-CSF. The HGFs are preferably in recombinant or synthetic form and, with the exception of the amino acid substitution(s) in the first α-helix, the amino acid sequence of the HGF may be the same as the naturally occurring molecule (i.e. native molecule) or may carry single or multiple amino acid substitutions, deletions and/or additions to the native amino acid sequence. The HGF sequences are preferably of mammalian origin such as from humans, livestock animals, companion animals or laboratory test animals. Most preferably, the HGFs are of human origin or of a mammalian origin capable of functioning in humans.

The first α-helix of GM-CSF has been determined at 2.4 angstrom resolution by X-ray crystallography and encompasses amino acid residues 13 to 28. Similar procedures may be adopted to determine the first α-helix in other haemopoietic growth factors. The position may also be determined by analogy to GM-CSF structure.

Reference to "unglycosylated form" herein means that the molecule is completely unglycosylated such as when expressed in recombinant form in a prokaryotic organism (e.g. *E. coli*). Alternatively, a glycosylation-deficient mammalian cell may be used or complete deglycosylation may occur *in vitro* using appropriate enzymes. Accordingly, the present invention extends to chemically synthesised GM-CSF which is in unglycosylated form.

An "exposed" amino acid is taken herein to refer to an amino acid on an exposed or outer portion of an α-helix compared to those amino acids orientated towards the inside of the molecule.

An acidic amino acid includes, for example, Glu and Asp. Preferred basic amino acids are Arg and Lys.

According to another aspect of the present invention, there is provided a haemopoietic growth factor characterised by:

(i) being in unglycosylated form;

(ii) comprising a sequence of amino acids within the first α-helix;

(iii) one or more exposed amino acids in said α-helix which have acidic or acidic-like properties being substituted by a basic amino acid residue;

(iv) being in recombinant or synthetic form;

(v) being capable of acting as an antagonist for at least one property of the corresponding native HGF.

This aspect of the present invention is predicated in part on the surprising discovery that a mutation in amino acid 21 (Glu) of hGM-CSF to Arg or Lys together with the variant in GM-CSF being in unglycosylated form results in the hGM-CSF variant being unable to detectably exhibit classical GM-CSF function. The variants, referred to herein "GM-CSF Arg^{21}" and "GM-CSF Lys^{21}" are unable to bind to high affinity receptors but are still able to fully bind the low affinity α chain of the GM-CSF receptor. Importantly, the non-glycosylated GM-CSF Arg^{21} and GM-CSF Lys^{21} act as antagonists, preventing the stimulatory effect of native GM-CSF. For convenience, the numbering of amino acid

residues in hGM-CSF is taken from Wong *et al.* (1985).

By way of a shorthand notation the following three letter abbreviations for amino acid residues are used in the specification as defined in Table 1.

Where a specific amino residue is referred to by its position in the polypeptide of an HGF, the amino acid abbreviation is used with the residue number given in superscript (i.e. Xaa^n, wherein Xaa is the amino acid residue).

Table 1

Amino acid	Three-letter abbreviation	Corresponding single-letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

The present invention is exemplified using GM-CSF and in particular hGM-CSF Arg^{21} and hGM-CSF Lys^{21}. This is done, however, with the understanding that the present invention extends to all HGFs as hereinbefore described.

For example, given that there is a Glu at position 22 of IL-3 and position 13 in IL-5 which in the three dimensional structure (in the case of IL-5) occupies an equivalent position to Glu^{21} of GM-CSF, then the present invention provides the basis for the creation of antagonists in IL-3 and IL-5. The substitution of Glu at these positions in IL-3 and IL-5 may be the sole mutation or it may be in combination with other amino acid mutations (including substitutions, deletions and/or additions) for the development of effective antagonists. This similarly applies to other HGFs based on the acidic or acidic-like amino acid residues in the first α-helix of the molecule. The location of the N-terminal helix can in each case be readily determined on comparable motifs from predicted helices. Such HGFs are listed in Table 2 showing the acidic or acidic-like amino acid residue in bold in the equivalent position to Glu^{21} of hGM-CSF. The acidic or acidic-like amino acid residues are readily substituted by, for example, recombinant DNA technology.

According to another aspect of the present invention there is provided a modified variant including HGF comprising an amino acid sequence in the first α-helix of said HGF selected from the group consisting of:

i) His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu (SEQ ID No. 1);
ii) Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu (SEQ ID No. 2);
iii) Asn Met Ile Xaa Xaa Ile Ile Thr His Leu (SEQ ID No. 3);
iv) Leu Leu Leu Xaa Leu Gln Met Ile Leu (SEQ ID No. 4);
v) Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu (SEQ ID No. 5);
vi) Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys (SEQ ID No. 6);
vii) Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile (SEQ ID No. 7);

viii) Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp (SEQ ID No. 8);

ix) Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser (SEQ ID No. 9);

x) Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile (SEQ ID No. 10); and

xi) Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly (SEQ ID No. 11);

wherein Xaa is a basic amino acid, preferably selected from the group consisting of Arg and Lys, and wherein said variant HGF is in unglycosylated form and acts as an antagonist for at least one property of the corresponding native HGF. Preferably, the haemopoietic growth factor is hGM-CSF and Xaa is Arg or Lys at position 21 of the first α-helix.

The HGF antagonists of the present invention and in particular GM-CSF Arg^{21} and GM-CSF Lys^{21} are useful *inter alia* in the treatment of myeloid and lymphocyte leukaemias, some tumours of non-haemopoietic origins and acute and chronic inflammation such as asthma and rheumatoid arthritis. These and other conditions are considered herein to result from or be facilitated by the aberrant effects of an endogenous HGF such as GM-CSF. hGM-CSF Arg^{21} and hGM-CSF Lys^{21} will also be useful in mobilising stem cells and progenitor cells into the circulation without the risk of activating neutrophils and monocytes. Other related molecules may have different useful properties.

The present invention, therefore, contemplates a method of treatment comprising the administration to a mammal of an effective amount of a modified HGF as hereinbefore defined and in particular hGM-CSF Arg^{21} or hGM-CSF Lys^{21} or both for a time and under conditions sufficient for effecting said treatment. Generally, the mammal is a human, livestock animal, companion animal or laboratory test animal. Most preferably, the mammal is a human. A single modified HGF may be administered or a combination of variants of the same HGF. For example, a range of hGM-CSF variants could be used such as a combination of hGM-CSF Arg^{21} and hGM-CSF Lys^{21}.

TABLE 2

Cytokines related to GM-CSF exhibit a conserved acidic residue analogous to E21 in GM-CSF

CYTOKINE[2]	HELIX[1] (Amino Acid Residue No.)	AMINO ACID SEQUENCE	SEQ ID No.
hGM-CSF	15-28	His Val Asn Ala Ile Gln **Glu** Ala Arg Arg Leu Leu Asn Leu	12
hIL-5	9-24	Ala Leu Val Lys **Glu** Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu	13
hIL-3	18-27	Asn Met Ile **Asp** **Glu** Ile Ile Thr His Leu	14
hIL-2	17-25	Leu Leu Leu **Asp** Leu Gln Met Ile Leu	15
hIL-4	8-17	Ile Thr Leu Gln **Asp** Ile Ile Lys Thr Leu	16
hIL-6	18-43	Arg Tyr Ile Leu **Asp** Gly Ile Ser Ala Leu Arg Lys	17
hIL-7	9-20	Gly Asp Gln Tyr **Glu** Ser Val Leu Met Val Ser Ile	18
hIL-9	7-22	Ala Gly Ile Leu **Asp** Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp	19
hIL-10	21-31	Asn Met Leu Arg **Asp** Leu Arg Asp Ala Phe Ser	20
hG-CSF	13-24	Phe Leu Leu Lys Cys Leu **Glu** Gln Val Arg Lys Ile	21
hEPO	4-28	Tyr Leu Leu Glu Ala Lys **Glu** Ala Glu Asn Ile Thr Thr Gly	22

1 Only the pertinent portion of each helix is shown.

2 Predicted helices are from: for IL-2, (Brandhuber *et al*, 1987; Zurawski and Zurawski, 1989); for hIL-3 (Parry *et al*, 1988); for mIL-5, (Parry *et al*, 1988); for hIL-6 (Bazan, 1990a); for hG-CSF, (Parry *et al*, 1988); for hEPO, (Bazan, 1990); for hGM-CSF the first helix was determined from the crystal structure (Karplus, 1991). The location of the N-terminal helix in the other cytokines was based on comparable motifs from these secondary structure predictions.

The present invention also provides a pharmaceutical composition comprising the variant HGFs as hereinbefore defined or combinations thereof. Most particularly, the pharmaceutical composition comprises hGM-CSF Arg^{21} or hGM-CSF Lys^{21} or both.

Methods for preparing pharmaceutical compositions are well known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, Mack Publishing Company, Eaton, Pennsylvania, USA and may also include one or more pharmaceutical acceptable carriers and/or diluents.

The present invention is further described by reference to the following non-limiting Examples and/or Figures.

In the Figures:

Figure 1 is a graphical representation showing titration of *E. coli* derived GM-CSF Arg^{21} for its ability to affect 0_2^- production in human neutrophils (□) and to antagonise the enhancement of 0_2^- by wild type GM-CSF tested at 1.0 ng/ml (△) and at 0.1 ng/ml (closed triangle).

Figure 2 is a graphical representation showing failure of *E. coli*-derived GM-CSF Arg^{21} to antagonise the enhancement of 0_2^- production in human neutrophils stimulated with tumour necrosis factor-α (TNF-α).

Figure 3 is a graphical representation showing competitive inhibition of ^{125}I-GM-CSF binding to COS cells transfected with the GM-CSF receptor α chain alone (top) or α and β chains (bottom) by GM-CSF Arg^{21}.

Figure 4 is a graphical representation showing titration of GM-CSF Arg^{21} for its ability to antagonise GM-CSF (A) in contrast to no effect on IL-3 (B)-mediated proliferation of TF-1 cells.

Figure 5 is a graphical representation showing the titration of GM-CSF Arg^{21} for its ability to antagonise TF-1 proliferation stimulated by either *E. coli*-derived GM-CSF (A), yeast-derived GM-CSF (B) or CHO-derived GM-CSF (C).

Figure 6 is a graphical representation showing the titration of GM-CSF Arg^{21} for its ability to antagonise three primary human myeloid leukaemia (A, B & C) *ex vivo*.

Figure 7 is a graphical representation showing that E21R antagonises both GM-CSF but not TNF-α-mediated stimulation of human neutrophils (A), and both E21R and E21K also antagonise neurtrophil stimulation by CHO cell-derived GM-CSF (B). In panel A, titrations of *E. coli*-derived wild type GM-CSF (●), TNF-α (◆) and E21R (■) are shown. In antagonistic experiments, E21R was titrated against 1ng/ml of *E. coli*-derived GM-CSF (□) or 3ng/ml TNF-α (◊). In panel B, titrations of CHO cell-derived wild type GM-CSF (○), E21R (■) and E21K (▲) are shown. In antagonistic experiments, E21R (□) or E21K (△) were titrated against 3ng/ml CHO cell-derived GM-CSF. Each value represents the mean of triplicate determinations and error bars represent the SEM.

EXAMPLE 1

Expression of wild type and GM-CSF Arg^{21} and GM-CSF Lys^{21} in an *E. coli* expressed system

Wild type GM-CSF was expressed in *E. coli* using a plasmid (designated pshGM-CSF) containing a synthetic human GM-CSF cDNA cloned into the *E. coli* expression vector pIN-III-OmpH3, a derivative of the vector pIN-III-OmpA2 (Ghrayeb *et al*, 1984). GME21R was expressed from the plasmid pSGM21.1 containing $Glu^{21} \rightarrow Arg^{21}$ substitution and was derived from the pSGM-CSF parental plasmid. GME21K was expressed from the plasmid pSGM21.4 containing $Glu^{21} \rightarrow Lys^{21}$ substitution and was derived from the pSGM-CSF parental plasmid.

pSGM21 was generated by initially eliminating a SacII site from the wild type GM-CSF using oligonucleotide cassette mutagenesis to generate plasmid pSGMV1. A 64 bp Nco 1/SacII fragment was then excised from the pSGMV1 plasmid and replaced by double-stranded 64bp oligonucleotides containing the appropriate mutation in the DNA sequence.

pSGM21.4 was generated by excising an 88 bp Bgl11/SacII fragment from pSGF1 and replacing it with 88bp oligonucleotides containing the appropriate mutation site directed mutagenesis (Zoller & Smith, 1984).

Protein was expressed in either MC1061, for wild type GM-CSF or BL21 for GME21R or GME21K, after induction by isopropyl β-D-thiogalactoside and recovered from the periplasmic space by osmotic shock (Koshland and Botstein, 1980).

GM-CSF protein was purified using a monoclonal antibody 4A12 generated in the laboratory coupled to Sepharose beads. Further purification was achieved by reversed phase HPLC using a BioRad controller and a Brownlee Aquaport C8 100 x 10mm column. GM-CSF was eluted using a 30-50% gradient of acetonitrile in 0.1% trifluoroacetic acid.

Resultant purified GM-CSF was lyophilised and resuspended in 1 x PBS before being quantitated by HPLC gel filtration. Samples were fractionated on a Beckman Ultraspherogel SEC3000 7.5 x 300mm using a 0.1M Na Phosphate pH 7.0/01M Na_2SO_4 mobile phase. Purity was estimated at >95% and area under peaks corresponding to GM-CSF integrated as the extinction coefficient of 0.95 absorbance units.ml.mg^{-1}.

EXAMPLE 2

Visualisation of mutant GM-CSF protein

GM-CSF unpurified or purified from *E. coli* was size-fractionated by $NaDodSo_4$/12.5% w/v polycarylamide gel electrophoresis (Laemmli, 1970). For Western blot analysis, protein was transferred to nitrocellulose as described (Towbin *et al,* 1979). Filters were probed with a sheep anti-GM-CSF followed by a second layer of biotinylated-rabbit anti-sheep IgG. After a further incubation with an avidin-biotinylated-horseradish peroxidase conjugate, the complex was visualised using a diaminobenzidine substrate solution. For silver staining, the method of Morrissey (1981) was used.

EXAMPLE 3

Stimulation of haemopoietic cell proliferation

The human erythroleukaemia cell line TF-1 (and myeloid leukaemia) cells were used to measure the proliferative function of GM-CSF and GM-CSF Arg^{21}. Proliferation of TF-1 cells were measured by the ability to incorporate [^{3}H]-thymidine in response to increasing doses of GM-CSF. This assay was performed as described by Lopez *et al* (1988).

EXAMPLE 4

Functional activation of human granulocytes and monocytes

The superoxide anion production assay was carried out as previously described (Lopez *et al,* 1986).

EXAMPLE 5

Radioreceptor assay

(a) Radioiodination of GM-CSF.

Yeast derived human GM-CSF or *E. coli*-derived human GM-CSF was radioiodinated by the ICl method (Contreras *et al*, 1983). Iodinated protein was separated from free ^{125}I by chromatography on a Sephadex G-25 PD10 column (Pharmacia, Uppsala, Sweden), equilibrated in phosphate buffered saline (PBS) containing 0.02% w/v Tween 20, and stored at 4°C for up to 4 weeks. Before use, the iodinated protein was purified from Tween and non-protein-associated radioactivity by cation exchange chromatography on a 0.3ml CM-Sepharose CL-6B column (Pharmacia) and stored at 4°C for up to 5 days. The radiolabelled GM-CSF retained >90% biological activity as judged from titration curves using non-iodinated GM-CSF as controls.

(b) Competition binding assays.

Competition for binding to high affinity and low affinity receptors used stably transfected CHO cell lines expressing either the α and β chains, or the α chain alone. The cells were suspended in binding medium consisting of RPMI-1640 supplemented with 20mmol/l HEPES and 0.5% w/v bovine serum albumin (BSA) and 0.1% w/v sodium azide. Typically, equal volumes (50μl) of 4 x 10^4 CHO cells, iodinated GM-CSF and different

concentrations of GM-CSF and GM-CSF Arg^{21} were mixed in siliconised glass tubes for 3 hr at 4°C. At the end of the incubation period, cell suspensions were overlaid on 0.2ml foetal calf serum (FCS) at 4°C, centrifuged in a Beckman Microfuge 12, and the tip of each tube containing the visible cell pellet cut off and counted in a gamma counter. Specific counts were determined by first subtracting the counts, obtained in the presence of excess wild type GM-CSF.

EXAMPLE 6

Generation of hGM-CSF Variants

By way of example only, the generation of GM-CSF Arg^{21} is hereinafter described in detail. A human GM-CSF cDNA was subjected to mutagenesis to introduce the amino acid Arg for Glu at position 21. Two mutants were obtained, one containing the $Glu^{21}\rightarrow$Arg mutation and a second one containing a double mutation $X^{10}\rightarrow$Ile and $Glu^{21}\rightarrow$Arg. These mutants were cloned into the expression system piN OMPIII and expressed in *E. coli*. Wild type (WT) GM-CSF was expressed in MC1061. GM-CSF Arg^{21} could not be expressed in MC1061. Of twenty strains tested for GM-CSF Arg^{21} expression, BL21 was the highest producer and used for subsequent studies.

To obtain purified GM-CSF Arg^{21} in high yields a two-step purification procedure was devised. In the first step, GM-CSF Arg^{21} was purified by an affinity column constructed with a monoclonal antibody (4A12) that binds to GM-CSF in solution and with high affinity. Affinity-purified GM-CSF Arg^{21} was then purified by reverse-phase HPLC and quantitated by HPLC before being analysed for biological and binding activities.

It was found that *E. coli*-derived GM-CSF Arg^{21} was unable to enhance neutrophil 0_2- production up to a concentration of 3,000 ng/ml (Figure 1). This is different to the inventors' previous results with CHO-derived (i.e. glycosylated) GM-CSF Arg^{21} which was able to enhance neutrophil function at approximately 30 ng/ml which represents a 300 fold reduced potency compared to wild type GM-CSF (Lopez *et al*, 1992).

Despite its inability to activate neutrophils, *E. coli*-derived GM-CSF Arg^{21} was able to bind as well as wild type GM-CSF to the α-chain of the GM-CSF receptor (Figure 3). In contrast, GM-CSF Arg^{21} exhibited an approximate 100-fold reduction in binding to the αβ GM-CSF receptor complex (Figure 3) indicating that the influence of the β chain has been selectively lost.

This is the first time that a GM-CSF mutant is shown to have unaltered binding to the GM-CSF receptor α-chain as well as being devoid of agonistic activity. This indicates that whilst binding to the α chain is necessary for GM-CSF activity it is not sufficient, and that GM-CSF binding to the β chain is required for GM-CSF-mediated activation.

Since *E. coli*-derived GM-CSF Arg^{21} was not able to stimulate neutrophils yet fully bound the GM-CSF receptor α chain, it was tested for antagonistic activity. The inventors found that this mutant fully inhibited the effect of wild type GM-CSF with an approximately 300-fold excess required to induce 50% inhibition of *E. coli*-derived wild type GM-CSF (Figure 1). This antagonistic effect was specific for GM-CSF as judged by the lack of antagonistic effect of GM-CSF Arg^{21} on TNF enhancement of neutrophil 0_2^- production (Figure 2).

The antagonistic effect of *E. coli*-derived GM-CSF Arg^{21} was present whether wild type GM-CSF was expressed in *E. coli* (Figure 5A), yeast (Figure 5B) or CHO cells (Figure 5C). In keeping with the differences in binding affinity between heavily glycosylated CHO GM-CSF (lower affinity), partially glycosylated yeast GM-CSF (intermediate affinity) and unglycosylated *E. coli* GM-CSF (higher affinity), *E. coli*-derived GM-CSF Arg^{21} antagonised better the CHO wild type GM-CSF (Figure 5).

The antagonism of *E. coli*-derived GM-CSF Arg^{21} was not restricted to proliferation of the established TF-1 cell line but was also seen in primary myeloid leukaemias. In three different leukaemias, *E. coli*-derived GM-CSF Arg^{21} antagonised the proliferative effect of wild type *E. coli* GM-CSF with an EC50 that varied with each leukaemia (Figure 6).

These results show that an unglycosylated GM-CSF molecule with a mutated Glu for an Arg in position 21 of the first α-helix is able to antagonise native GM-CSF.

Since the mutated Glu in GM-CSF is in a position where the same acidic residue or similar acidic residues (e.g. Asp) are present in related growth factors (see Table 2), this invention extends to antagonistic molecules for these growth factors constructed by incorporating the analogous charge reversal mutation. In particular, given that the GM-CSF, IL-3 and IL-5 receptor share the β chain, the Glu^{22} in IL-3 and the Glu^{13} in IL-5 are predicted to play a similar role. Other variant HGFs are shown in Examples 3 to 22 in which the equivalent or similar amino acid residue to Glu^{21} of hGM-CSF is replaced by either Arg or Lys. In these Examples, the amino acid sequences are provided for the relevant portion of the first α-helix carrying the substitution (see Table 2).

EXAMPLE 7

hGM-CSF Lys^{21}

His Val Asn Ala Ile Gln **Lys** Ala Arg Arg Leu Leu Asn Leu (SEQ ID NO. 23)

EXAMPLE 8

hIL-5 Lys^{13}

Ala Leu Val Lys **Lys** Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu (SEQ ID NO.24)

EXAMPLE 9

hIL-5 Arg^{13}

Ala Leu Val Lys **Arg** Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu (SEQ ID NO. 25)

EXAMPLE 10

hIL-3 Variants

Asn Met Ile **Asp Lys** Ile Ile Thr His Leu	hIL-3 Lys^{22}	(SEQ ID NO. 26)
Asn Met Ile **Lys Glu** Ile Ile Thr His Leu	hIL-3 Lys^{21}	(SEQ ID NO. 27)
Asn Met Ile **Asp Arg** Ile Ile Thr His Leu	hIL-3 Arg^{22}	(SEQ ID NO. 28)
Asn Met Ile **Arg Glu** Ile Ile Thr His Leu	hIL-3 Arg^{21}	(SEQ ID NO. 29)
Asn Met Ile **Lys Lys** Ile Ile Thr His Leu	hIL-3 Lys^{21} Lys^{22}	(SEQ ID NO. 30)

Asn Met Ile **Arg Arg** Ile Ile Thr His Leu hIL-3 Arg^{21} Arg^{22} (SEQ ID NO. 31)

EXAMPLE 11

hIL-2 Lys^{20}

Leu Leu Leu **Lys** Leu Gln Met Ile Leu (SEQ ID NO. 32)

EXAMPLE 12

hIL-2 Arg^{20}

Leu Leu Leu **Arg** Leu Gln Met Ile Leu (SEQ ID NO. 33)

EXAMPLE 13

hIL-4 Lys^{12}

Ile Thr Leu Gln **Lys** Ile Ile Lys Thr Leu (SEQ ID NO. 34)

EXAMPLE 14

hIL-4 Arg^{12}

Ile Thr Leu Gln **Arg** Ile Ile Lys Thr Leu (SEQ ID NO. 35)

EXAMPLE 15

hIL-6 Lys^{22}

Arg Tyr Ile Leu **Lys** Gly Ile Ser Ala Leu Arg Lys (SEQ ID NO. 36)

EXAMPLE 16

hIL-6 Arg^{22}

Arg Tyr Ile Leu **Arg** Gly Ile Ser Ala Leu Arg Lys (SEQ ID NO. 37)

EXAMPLE 17

hIL-7 Lys^{13}

Gly Asp Gln Tyr **Lys** Ser Val Leu Met Val Ser Ile (SEQ ID NO. 38)

EXAMPLE 18

hIL-7 Arg^{13}

Gly Asp Gln Tyr **Arg** Ser Val Leu Met Val Ser Ile (SEQ ID NO. 39)

EXAMPLE 19

hIL-9 Lys^{11}

Ala Gly Ile Leu **Lys** Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp (SEQ ID NO. 40)

EXAMPLE 20

hIL-9 Arg^{11}

Ala Gly Ile Leu **Arg** Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp (SEQ ID NO. 41)

EXAMPLE 21

hIL-10 Lys^{25}

Asn Met Leu Arg **Lys** Leu Arg Asp Ala Phe Ser (SEQ ID NO. 42)

EXAMPLE 22

hIL-10 Arg^{25}

Asn Met Leu Arg **Arg** Leu Arg Asp Ala Phe Ser (SEQ ID NO. 43)

EXAMPLE 23

hG-CSF Lys^{19}

Phe Leu Leu Lys Cys Leu **Lys** Gln Val Arg Lys Ile (SEQ ID NO. 44)

EXAMPLE 24

hG-CSF Arg^{19}

Phe Leu Leu Lys Cys Leu **Arg** Gln Val Arg Lys Ile (SEQ ID NO. 45)

EXAMPLE 25

hEPO Lys^{10}

Tyr Leu Leu Glu Ala Lys **Lys** Ala Glu Asn Ile Thr Thr Gly (SEQ ID NO. 46)

EXAMPLE 26

hEPO Arg^{10}

Tyr Leu Leu Glu Ala Lys **Arg** Ala Glu Asn Ile Thr Thr Gly (SEQ ID NO. 47)

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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(2) INFORMATION FOR SEQ ID NO. 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acid residues
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu

(2) INFORMATION FOR SEQ ID NO. 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

Asn Met Ile Xaa Xaa Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 4:

Leu Leu Leu Xaa Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 5:

Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 6:

Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 7:

Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 8:

Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 9:

Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 10:

Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 11:

Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly

(2) INFORMATION FOR SEQ ID NO. 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 12:

His Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu

(2) INFORMATION FOR SEQ ID NO. 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 13:

Ala Leu Val Lys Glu Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

Asn Met Ile Asp Glu Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 15:

Leu Leu Leu Asp Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 16:

Ile Thr Leu Gln Asp Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 17:

Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 18:

Gly Asp Gln Tyr Glu Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 19:

Ala Gly Ile Leu Asp Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 20:

Asn Met Leu Arg Asp Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 21:

Phe Leu Leu Lys Cys Leu Glu Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 22:

Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly

(2) INFORMATION FOR SEQ ID NO. 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 23:

His Val Asn Ala Ile Gln Lys Ala Arg Arg Leu Leu Asn Leu

(2) INFORMATION FOR SEQ ID NO. 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 24:

Ala Leu Val Lys Lys Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 25:

Ala Leu Val Lys Arg Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu

(2) INFORMATION FOR SEQ ID NO. 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 26:

Asn Met Ile Asp Lys Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 27:

Asn Met Ile Lys Glu Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 28:

Asn Met Ile Asp Arg Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 29:

Asn Met Ile Arg Glu Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 30:

Asn Met Ile Lys Lys Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 31:

Asn Met Ile Arg Arg Ile Ile Thr His Leu

(2) INFORMATION FOR SEQ ID NO. 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 32:

Leu Leu Leu Lys Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 33:

Leu Leu Leu Arg Leu Gln Met Ile Leu

(2) INFORMATION FOR SEQ ID NO. 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 34:

Ile Thr Leu Gln Lys Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 35:

Ile Thr Leu Gln Arg Ile Ile Lys Thr Leu

(2) INFORMATION FOR SEQ ID NO. 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 36:

Arg Tyr Ile Leu Lys Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 37:

Arg Tyr Ile Leu Arg Gly Ile Ser Ala Leu Arg Lys

(2) INFORMATION FOR SEQ ID NO. 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 38:

Gly Asp Gln Tyr Lys Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 39:

Gly Asp Gln Tyr Arg Ser Val Leu Met Val Ser Ile

(2) INFORMATION FOR SEQ ID NO. 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 40:

Ala Gly Ile Leu Lys Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 41:

Ala Gly Ile Leu Arg Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp

(2) INFORMATION FOR SEQ ID NO. 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 42:

Asn Met Leu Arg Lys Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 43:

Asn Met Leu Arg Arg Leu Arg Asp Ala Phe Ser

(2) INFORMATION FOR SEQ ID NO. 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 44:

Phe Leu Leu Lys Cys Leu Lys Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 45:

Phe Leu Leu Lys Cys Leu Arg Gln Val Arg Lys Ile

(2) INFORMATION FOR SEQ ID NO. 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 46:

Tyr Leu Leu Glu Ala Lys Lys Ala Glu Asn Ile Thr Thr Gly

(2) INFORMATION FOR SEQ ID. NO. 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO. 47:

Tyr Leu Leu Glu Ala Lys Arg Ala Glu Asn Ile Thr Thr Gly

CLAIMS:

1. A modified haemopoietic growth factor (HGF) characterised by being in unglycosylated form and comprising a sequence of amino acids within a first α-helix wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue.

2. A modified HGF according to claim 1, wherein said HGF is a modified form of an HGF selected from granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL) -2, IL-3, IL-4, IL-5, IL-6, Il-7, IL-9, IL-10, G-CSF, erythropoietin (EPO).

3. A modified HGF according to claim 2 wherein said HGF is a modified form of GM-CSF.

4. A modified HGF according to claim 1 or 2 or 3 wherein said HGF is of human, livestock animal, companion animal or laboratory test animal origin.

5. A modified HGF according to claim 4 wherein said HGF is of human origin.

6. A modified HGF according to any one of the preceding claims wherein the acidic amino acid residue on the first α-helix is Glu and/or Asp and the basic amino acid residue substituted therefor is Arg and/or Lys.

7. A modified HGF comprising an amino acid sequence in the first α-helix of said HGF selected from the group consisting of:

i) His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu;
ii) Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu;
iii) Asn Met Ile Xaa Xaa Ile Ile Thr His Leu;
iv) Leu Leu Leu Xaa Leu Gln Met Ile Leu;
v) Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu;

vi) Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys;
vii) Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile;
viii) Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp;
ix) Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser;
x) Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile;
xi) Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly;

wherein Xaa is a basic amino acid, selected from the group consisting of Arg and Lys and wherein said modified HGF is in unglycosylated form and acts as an antagonist for at least one property of the corresponding native HGF.

8. A modified HGF according to claim 7 wherein the HGF is a modified form of an HGF selected from the list consisting of GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, Il-7, IL-9, IL-10, G-CSF, EPO.

9. A modified HGF according to claim 8, wherein the variant HGF is a modified form of GM-CSF.

10. A modified HGF according to claim 7 or 8 wherein Xaa is Arg.

11. A modified human GM-CSF in unglycosylated form having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Arg Ala Arg Arg Leu Leu Asn Leu.

12. A modified human GM-CSF in unglycosylated form having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Lys Ala Arg Arg Leu Leu Asn Leu.

13. A modified human IL-5 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ala Leu Val Lys Lys Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu.

14. A modified human IL-5 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ala Leu Val Lys Arg Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu.

15. A modified human IL-3 in unglycosylated form having an amino acid sequence in a first α-helix selected from the list consisting of:

Asn Met Ile Asp Lys Ile Ile Thr His Leu;
Asn Met Ile Lys Glu Ile Ile Thr His Leu;
Asn Met Ile Asp Arg Ile Ile Thr His Leu;
Asn Met Ile Arg Glu Ile Ile Thr His Leu;
Asn Met Ile Lys Lys Ile Ile Thr His Leu; and
Asn Met Ile Arg Arg Ile Ile Thr His Leu.

16. A modified human IL-2 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Leu Leu Leu Lys Leu Gln Met Ile Leu.

17. A modified human IL-2 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Leu Leu Leu Arg Leu Gln Met Ile Leu.

18. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ile Thr Leu Gln Lys Ile Ile Lys Thr Leu.

19. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ile Thr Leu Gln Arg Ile Ile Lys Thr Leu.

20. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Arg Tyr Ile Leu Lys Gly Ile Ser Ala Leu Arg Lys.

21. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Arg Tyr Ile Leu Arg Gly Ile Ser Ala Leu Arg Lys.

22. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Gly Asp Gln Tyr Lys Ser Val Leu Met Val Ser Ile.

23. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Gly Asp Gln Tyr Arg Ser Val Leu Met Val Ser Ile.

24. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Ala Gly Ile Leu Lys Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp.

25. A modified human IL-4 having an amino acid sequence in a first α-helix comprising
Ala Gly Ile Leu Arg Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp.

26. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Asn Met Leu Arg Lys Leu Arg Asp Ala Phe Ser.

27. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Asn Met Leu Arg Arg Leu Arg Asp Ala Phe Ser.

28. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Phe Leu Leu Lys Cys Leu Lys Gln Val Arg Lys Ile.

29. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Phe Leu Leu Lys Cys Leu Arg Gln Val Arg Lys Ile.

30. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Tyr Leu Leu Glu Ala Lys Lys Ala Glu Asn Ile Thr Thr Gly.

31. A modified human IL-4 in unglycosylated form having an amino acid sequence in a first α-helix comprising
Tyr Leu Leu Glu Ala Lys Arg Ala Glu Asn Ile Thr Thr Gly.

32. A method of ameliorating the aberrant effects of an endogenous HGF in a mammal, said method comprising administering to said mammal an effective amount of a modified HGF characterised by being in unglycosylated form and comprising a sequence of amino acids within a first α-heliz wherein one or more exposed amino acids in said first α-helix having acidic or acidic-like properties are substituted with a basic amino acid residue.

33. A method according to claim 32 wherein the modified HGF is selected from the group consisting of:

i) His Val Asn Ala Ile Gln Xaa Ala Arg Arg Leu Leu Asn Leu;
ii) Ala Leu Val Lys Xaa Thr Leu Ala Leu Leu Ser Thr His Arg Thr Leu;
iii) Asn Met Ile Xaa Xaa Ile Ile Thr His Leu;
iv) Leu Leu Leu Xaa Leu Gln Met Ile Leu;
v) Ile Thr Leu Gln Xaa Ile Ile Lys Thr Leu;
vi) Arg Tyr Ile Leu Xaa Gly Ile Ser Ala Leu Arg Lys;
vii) Gly Asp Gln Tyr Xaa Ser Val Leu Met Val Ser Ile;

viii) Ala Gly Ile Leu Xaa Ile Asn Phe Leu Ile Asn Lys Met Gln Glu Asp;

ix) Asn Met Leu Arg Xaa Leu Arg Asp Ala Phe Ser;

x) Phe Leu Leu Lys Cys Leu Xaa Gln Val Arg Lys Ile; and

xi) Tyr Leu Leu Glu Ala Lys Xaa Ala Glu Asn Ile Thr Thr Gly;

wherein Xaa is a basic amino acid selected from the group consisting of Arg and Lys and wherein said variant HGF is in unglycosylated form and acts as an antagonist for at least one property of the corresponding native HGF.

34. A method according to claim 32 or 33 wherein the HGF is a modified form of an HGF selected from GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, Il-7, IL-9, IL-10, G-CSF, EPO.

35. A method according to claim 32 or 33 wherein the modified HGF is a modified human GM-CSF having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Arg Ala Arg Arg Leu Leu Asn Leu.

36. A method according to claim 32 or 33 wherein the modified HGF is a modified human GM-CSF having an amino acid sequence in a first α-helix comprising His Val Asn Ala Ile Gln Lys Ala Arg Arg Leu Leu Asn Leu.

37. Use of one or more modified HGFs each as defined in claim 1 or 7 in the manufacture of a medicament for the treatment of the affects of an aberrant endogenous HGF.

38. An agent comprising one or more modified HGFs each as defined in claim 1 or 7 for treating the affects of an aberrant endogenous HGF.

39. A pharmaceutical composition comprising one or more modified HGFs each as defined in claim 1 or 7 together with one or more pharmaceutically acceptable carriers and/or diluents.

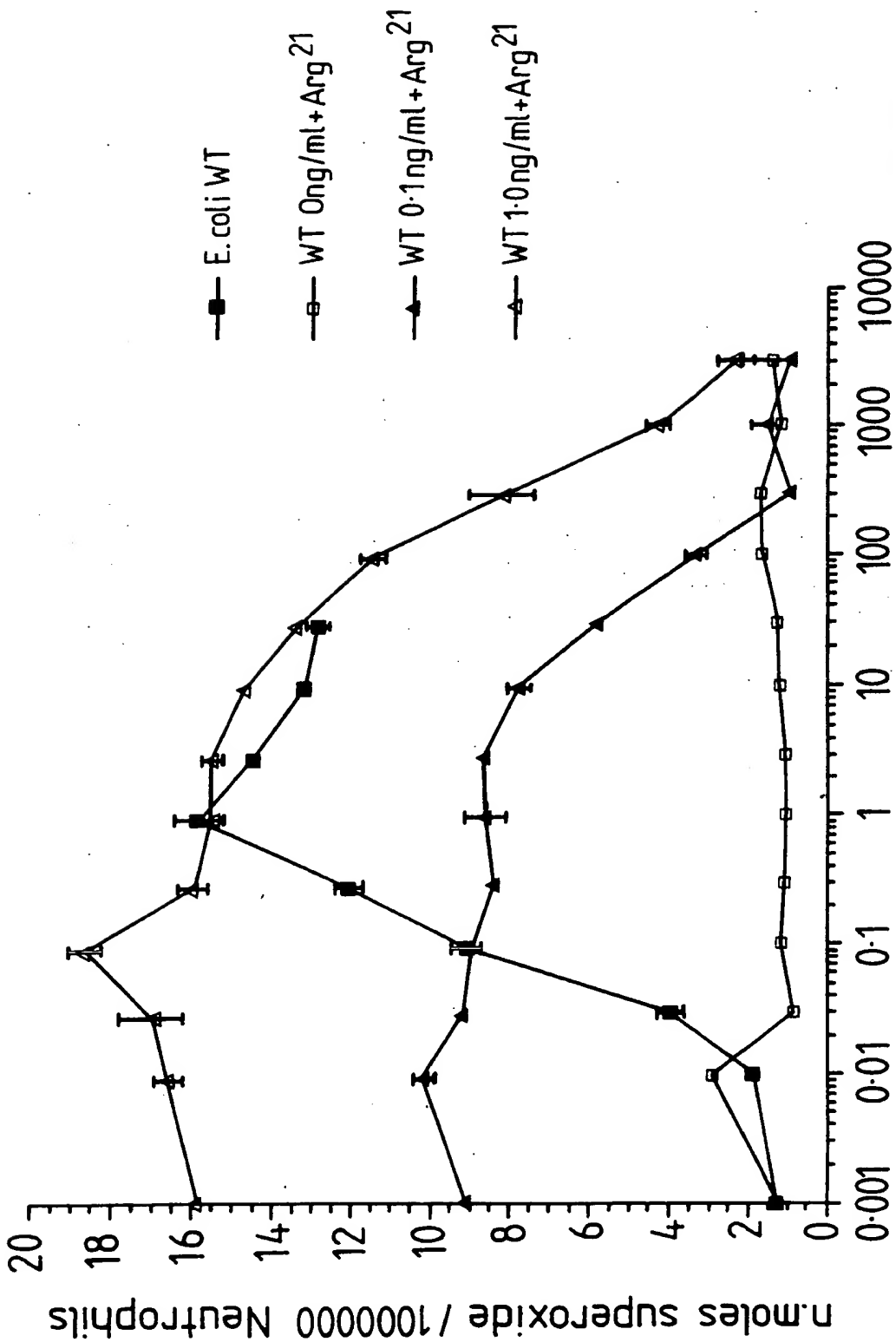

n.moles superoxide / 100000 Neutrophils
20
18
16
14
12
10
8
6
4
2
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)
E.coli WT
WT 0ng/ml+Arg21
WT 0·1ng/ml+Arg21
WT 1·0ng/ml+Arg21

FIGURE 1

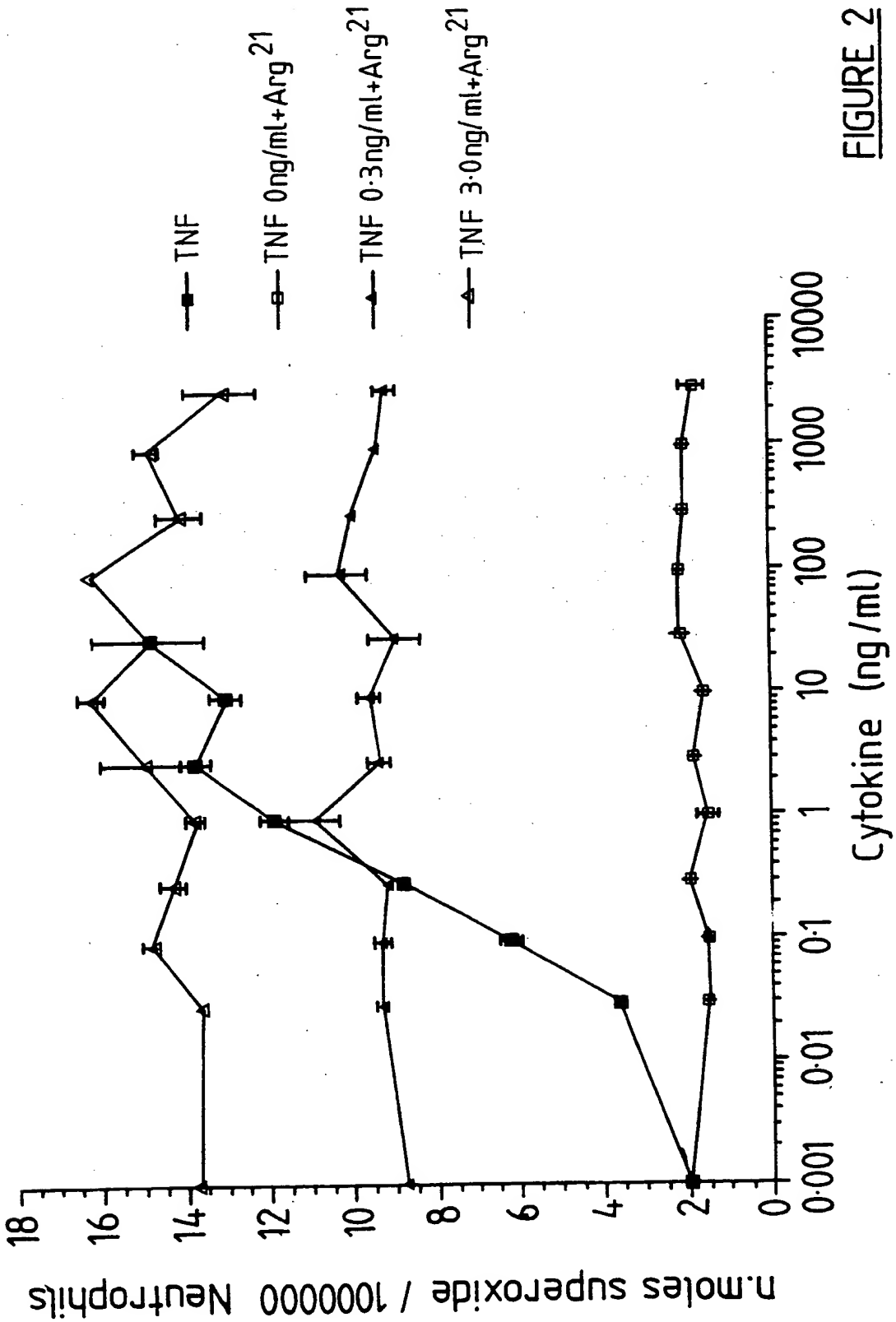
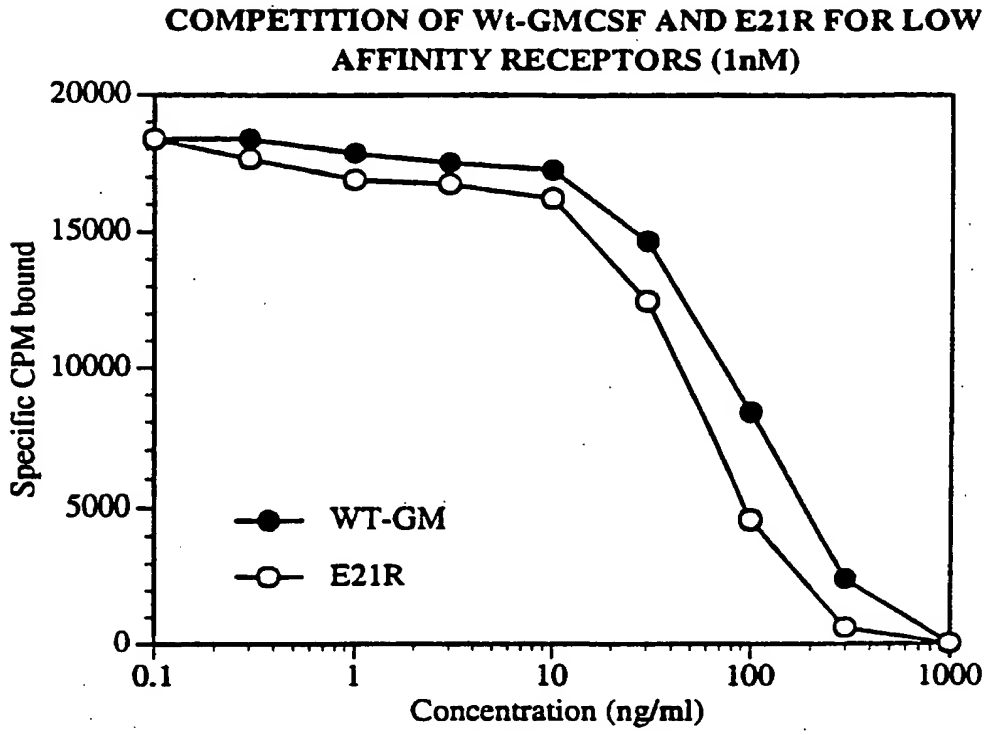

n.moles superoxide / 100000 Neutrophils
18
16
14
12
10
8
6
4
2
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)
TNF
TNF 0ng/ml+Arg21
TNF 0·3ng/ml+Arg21
TNF 3·0ng/ml+Arg21

FIGURE 2


COMPETITION OF Wt-GMCSF AND E21R FOR LOW AFFINITY RECEPTORS (1nM)
Specific CPM bound
20000
15000
10000
5000
0
0.1
1
10
100
1000
Concentration (ng/ml)
WT-GM
E21R

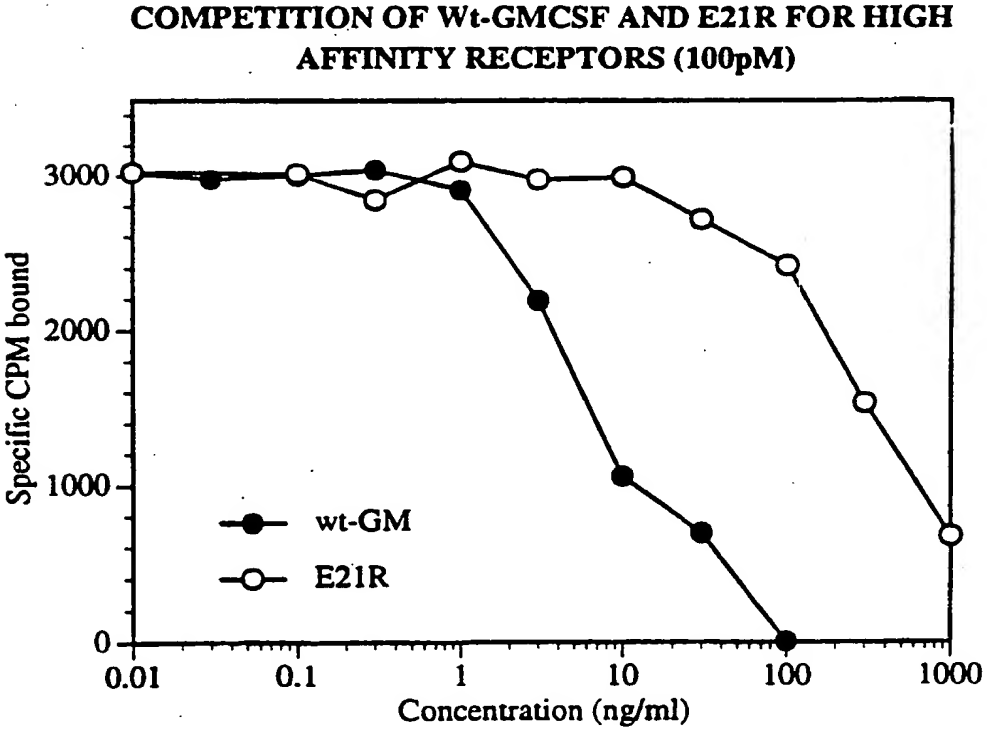

COMPETITION OF Wt-GMCSF AND E21R FOR HIGH AFFINITY RECEPTORS (100pM)
Specific CPM bound
3000
2000
1000
0
0.01
0.1
1
10
100
1000
Concentration (ng/ml)
wt-GM
E21R

FIGURE 3

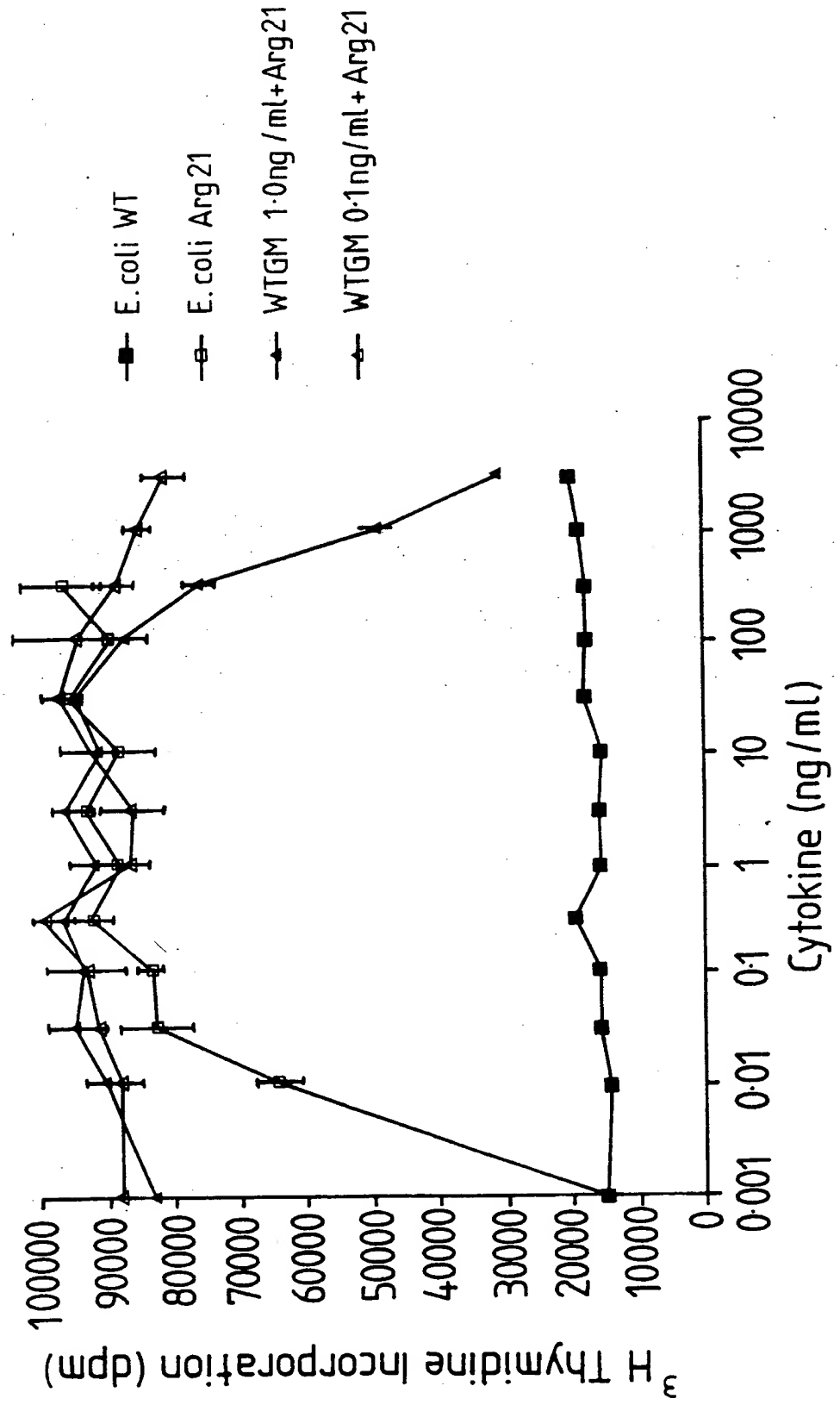

E.coli WT
E.coli Arg21
WTGM 1·0ng/ml+Arg21
WTGM 0·1ng/ml+Arg21
^{3}H Thymidine incorporation (dpm)
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)

FIG 4A

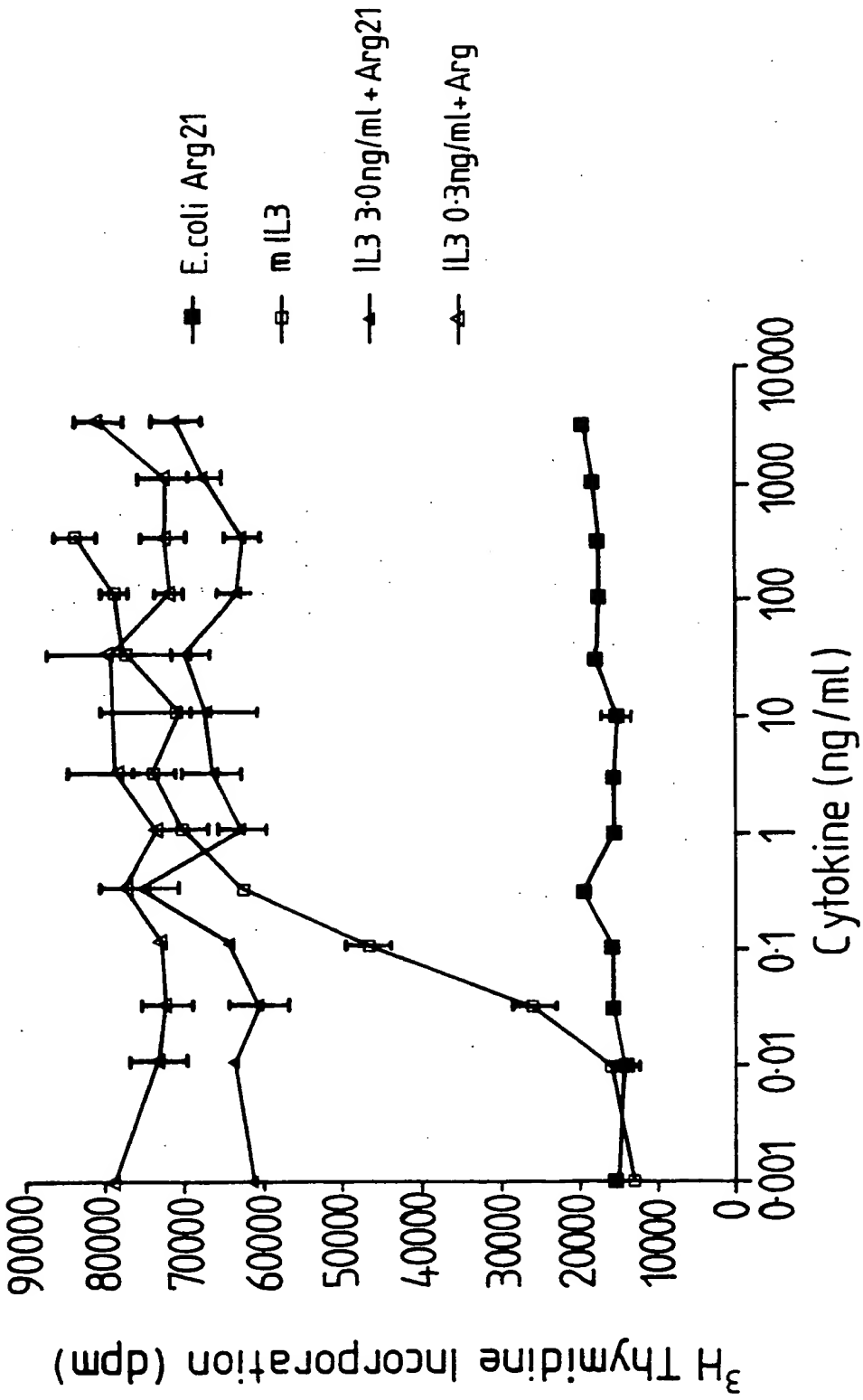

E.coli Arg21
m IL3
IL3 3·0ng/ml + Arg21
IL3 0·3ng/ml + Arg
^{3}H Thymidine incorporation (dpm)
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
0·001
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)

FIG 4B

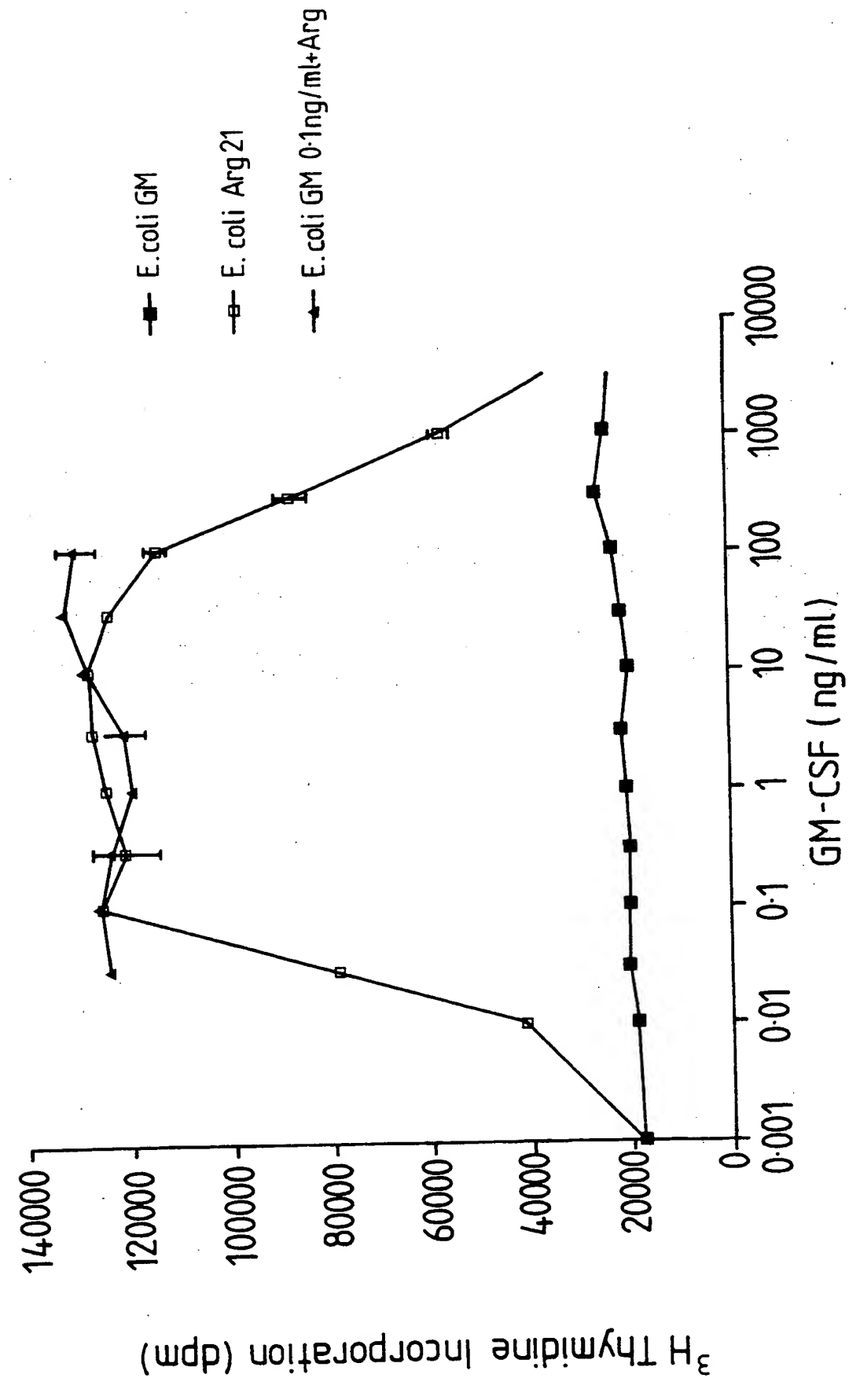

E.coli GM
E. coli Arg21
E.coli GM 0·1ng/ml+Arg
^{3}H Thymidine Incorporation (dpm)
140000
120000
100000
80000
60000
40000
20000
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 5A

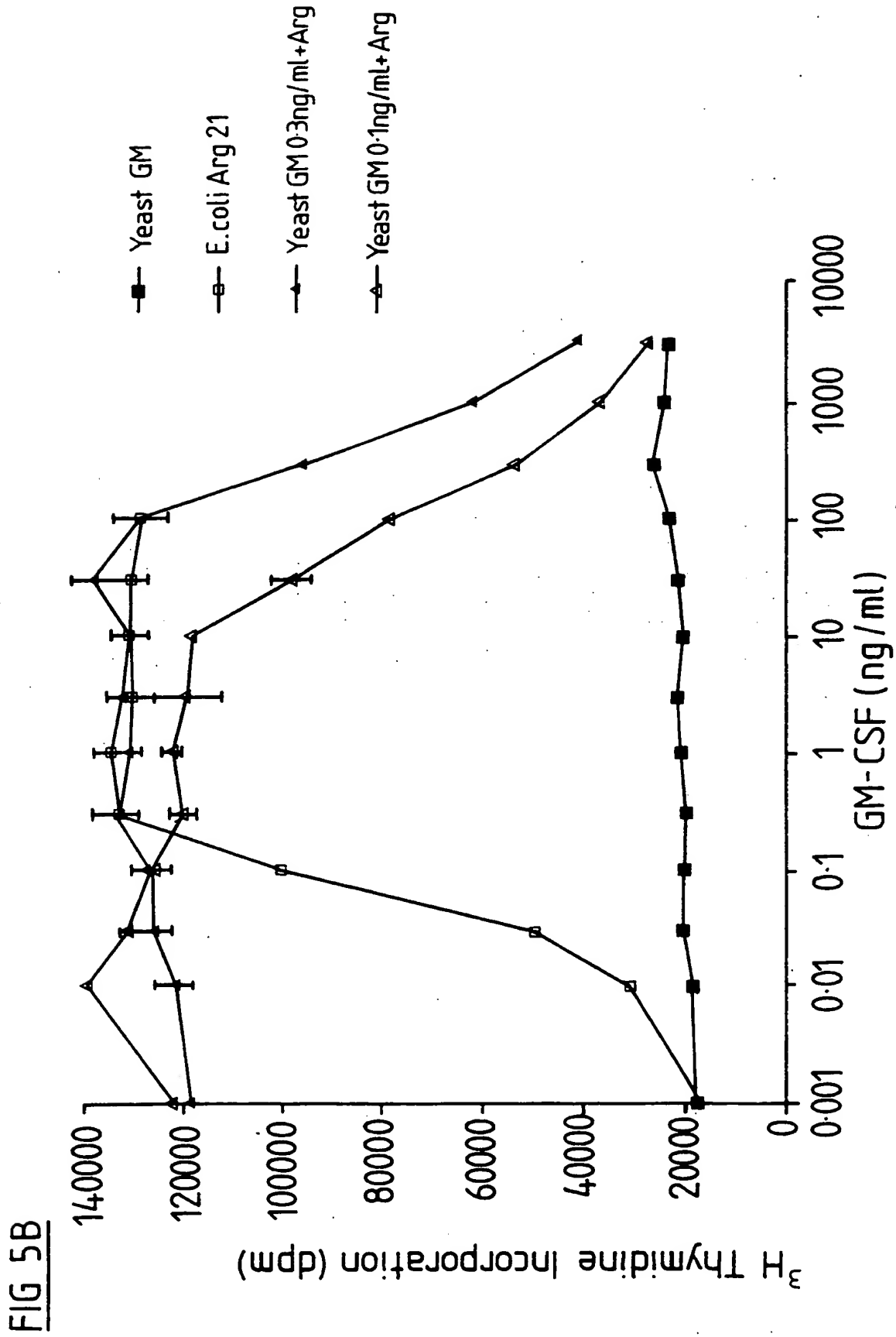

Yeast GM
E.coli Arg 21
Yeast GM 0·3ng/ml+Arg
Yeast GM 0·1ng/ml+Arg
^{3}H Thymidine Incorporation (dpm)
140000
120000
100000
80000
60000
40000
20000
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 5B

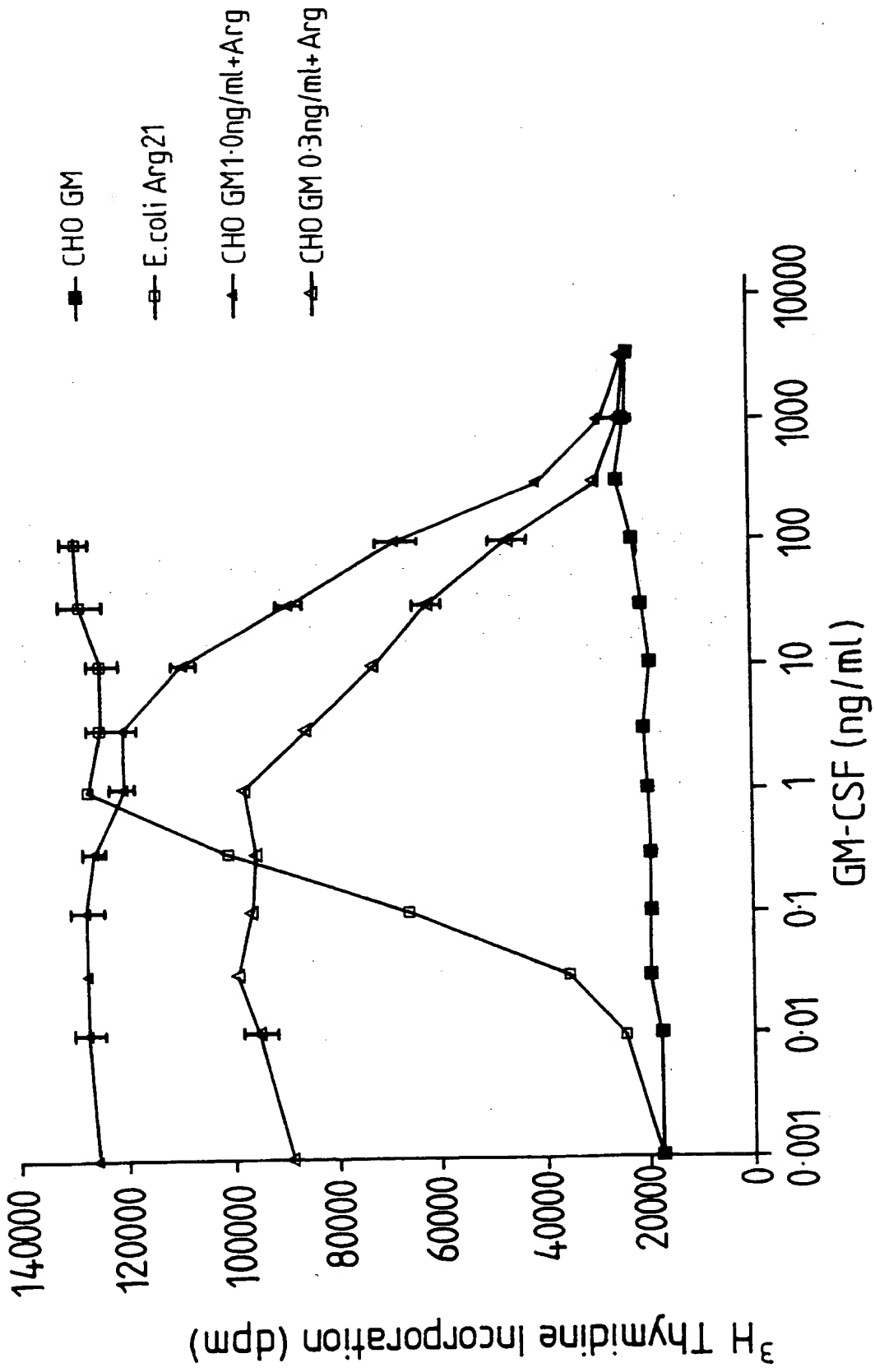

CHO GM
E.coli Arg21
CHO GM1·0ng/ml+Arg
CHO GM 0·3ng/ml+Arg
^{3}H Thymidine incorporation (dpm)
140000
120000
100000
80000
60000
40000
20000
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 5C

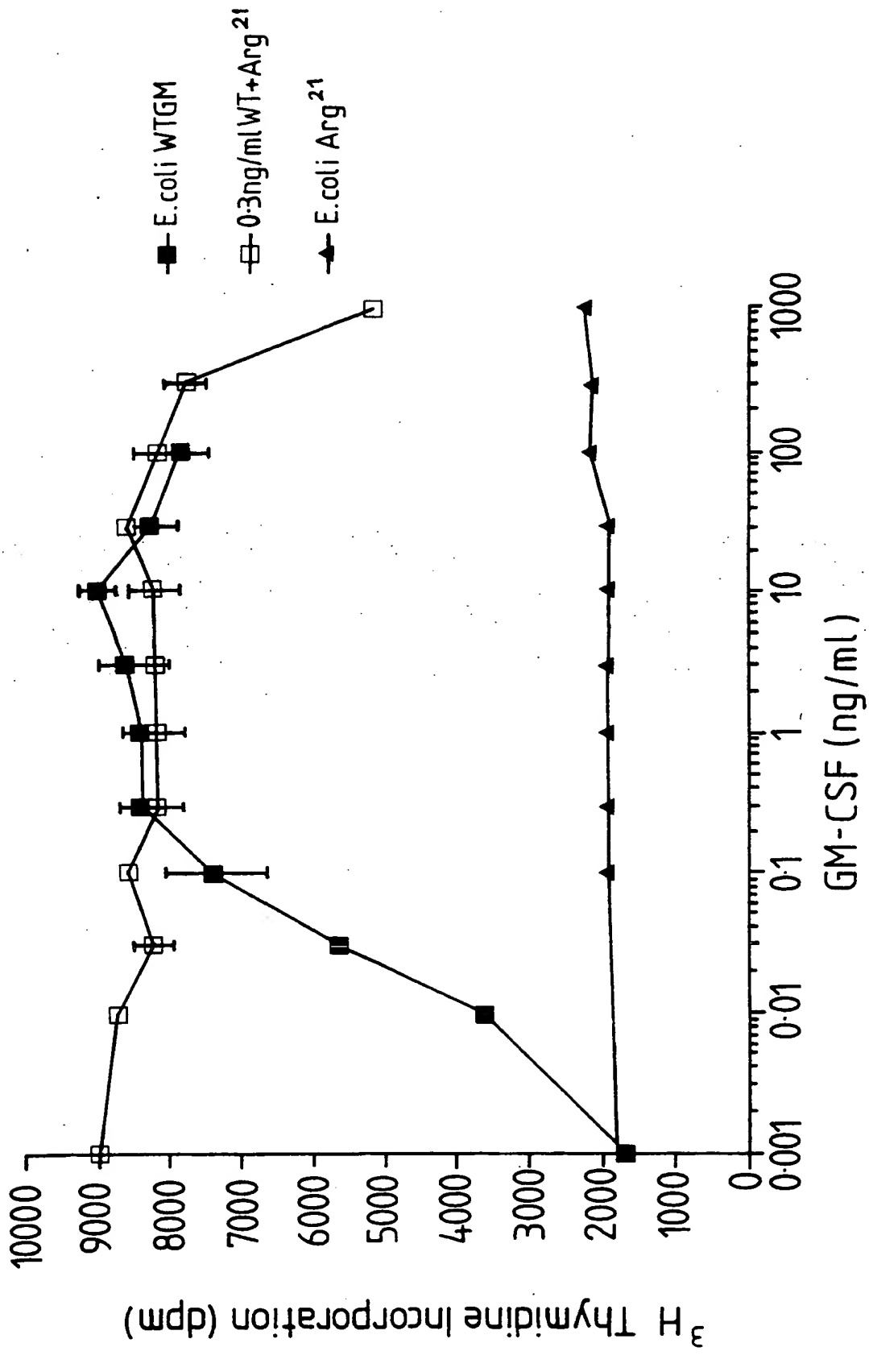

E.coli WTGM
0·3ng/ml WT+Arg21
E.coli Arg21
^{3}H Thymidine incorporation (dpm)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0·001
0·01
0·1
1
10
100
1000
GM-CSF (ng/ml)

FIG 6A

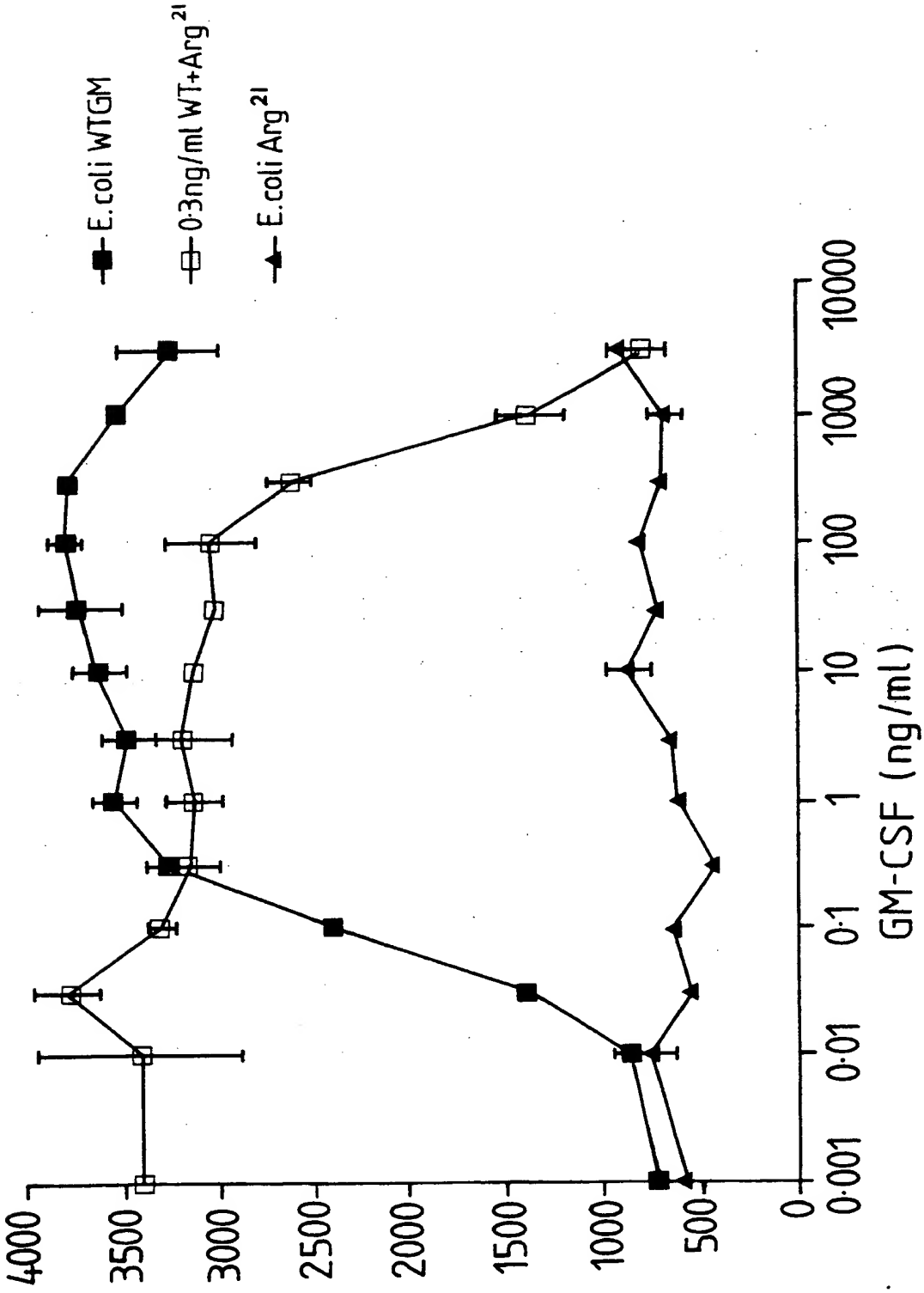

E.coli WTGM
0·3ng/ml WT+Arg21
E.coli Arg21
3H Thymidine incorporation (dpm)
4000
3500
3000
2500
2000
1500
1000
500
0
0·001
0·01
0·1
1
10
100
1000
10000
GM-CSF (ng/ml)

FIG 6B

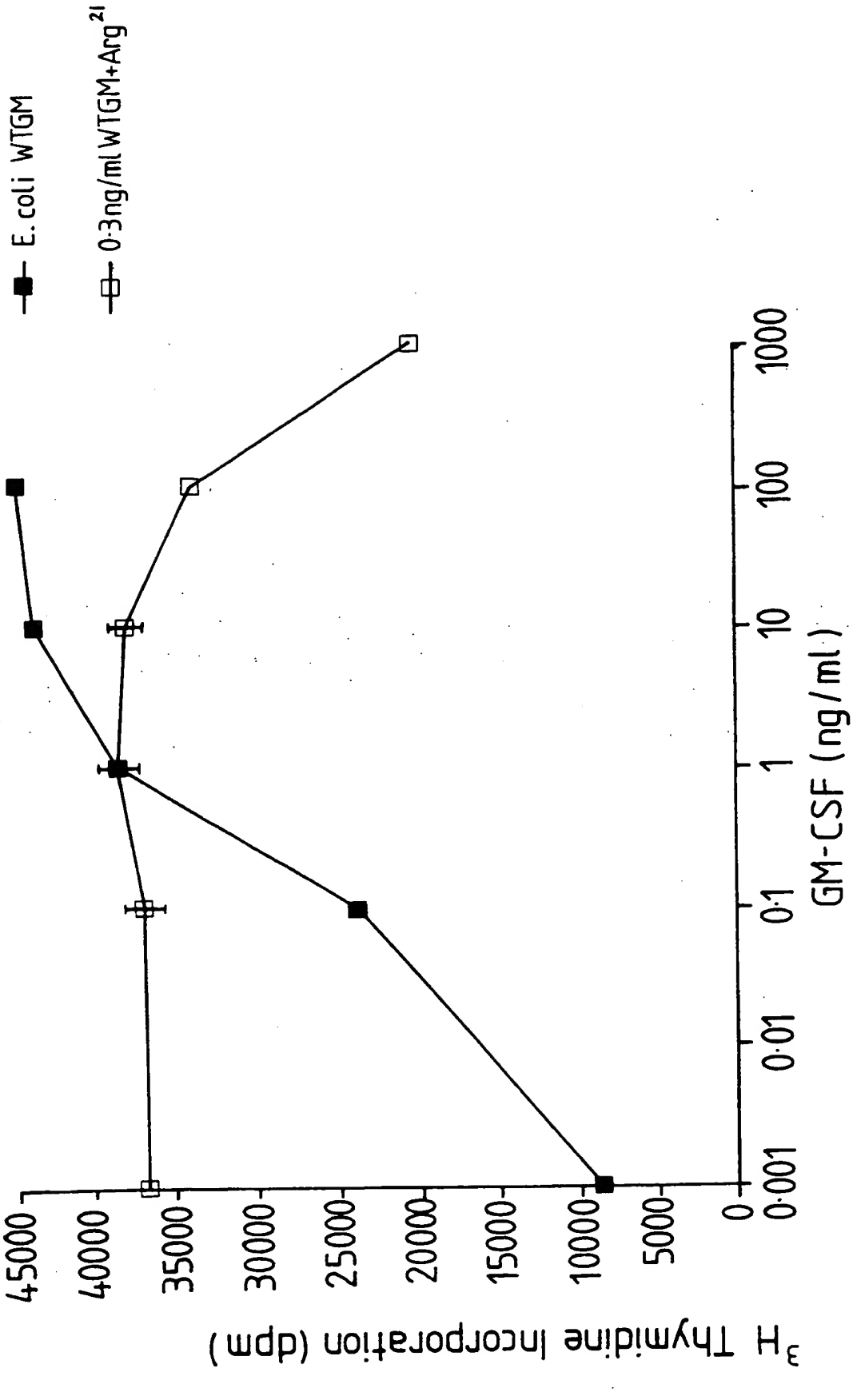

E. coli WTGM
0·3ng/ml WTGM+Arg21
3H Thymidine Incorporation (dpm)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0·001
0·01
0·1
1
10
100
1000
GM-CSF (ng/ml)

FIG 6C

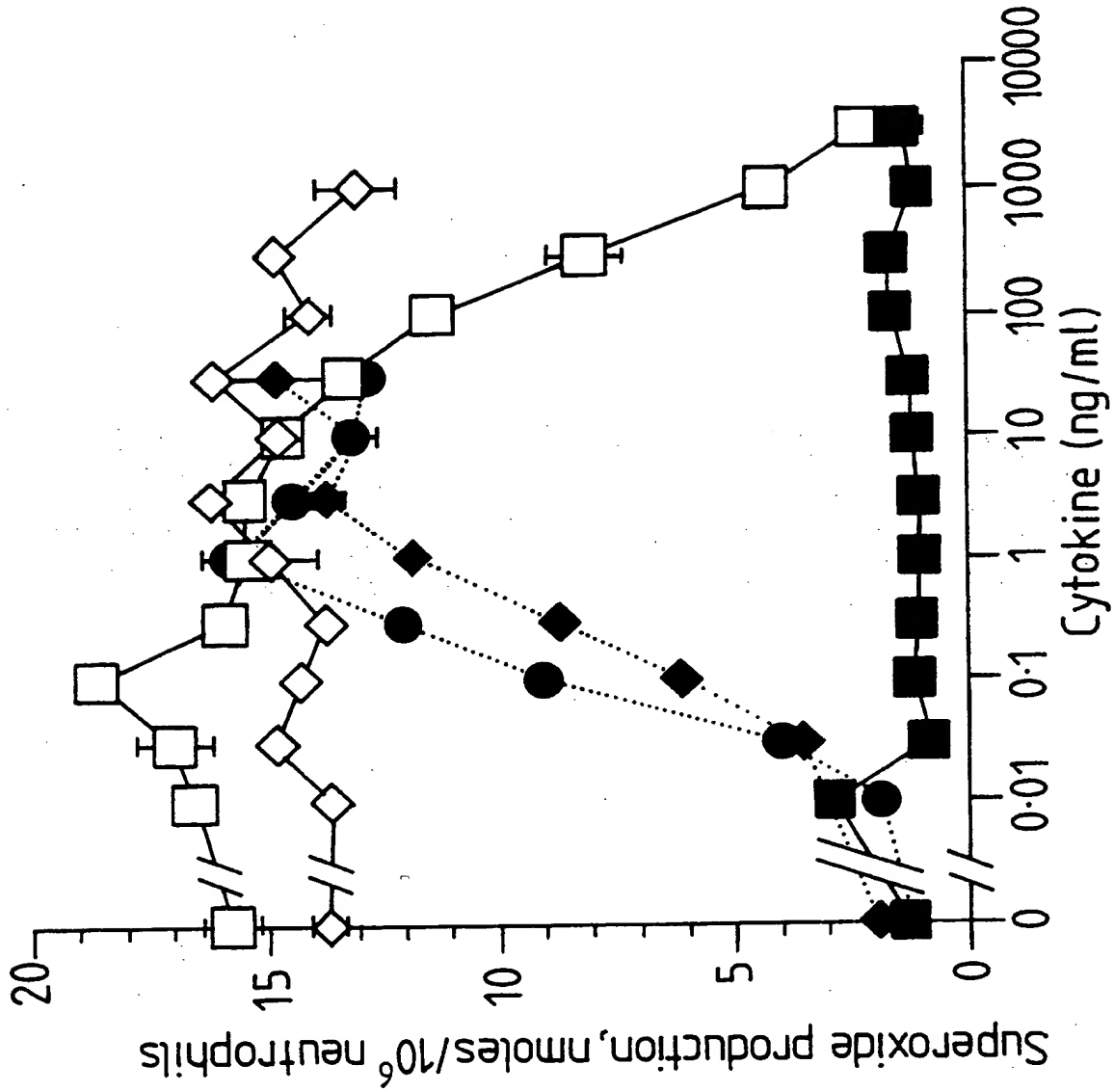

Superoxide production, nmoles/10^6 neutrophils
20
15
10
5
0
0
0·01
0·1
1
10
100
1000
10000
Cytokine (ng/ml)

FIG 7A

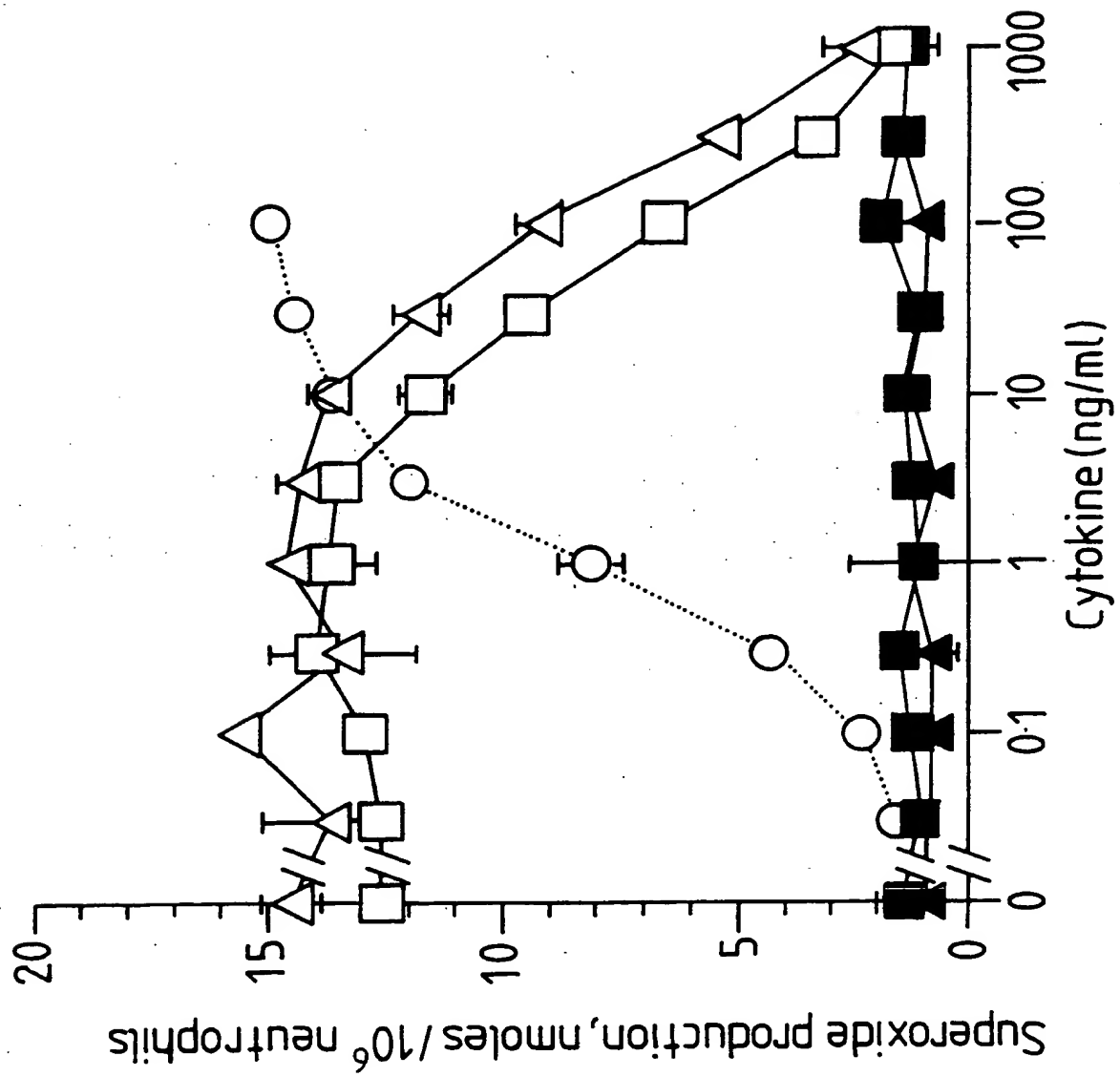

Superoxide production, nmoles / 10^6 neutrophils
20
15
10
5
0
0
0·1
1
10
100
1000
Cytokine (ng/ml)

FIG 7B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 94/00432

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[6] C07K 14/52, 14/505, 14/535, 14/54, 14/55, 14/475, C12N 15/19, 15/24, 15/26, 15/27, C12P 21/02, A61K 38/18, 38/19, 38/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Derwent database; World Patent Index (WPAT); American Chemical Society database; Chemical Abstracts (CA). See "electronic data base" box below for key words used.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC: AU C12N 15/19, 15/24, 15/27, 15/26

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)

WPAT, CA, DERWENT BIOTECHNOLOGY ABSTRACTS DATABASE (BIOT): (KEYWORDS) MUTEIN#; VARIANT#; ANALOG:; COLONY STIMULATING FACTOR#; INTERLEUKIN#; ERYTHROPOIETIN#; HELIC:; HELIX:

Protein sequences - see Supplemental Box

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	AU,B,60960/90 (636641) (GIST-BROCADES N.V.) 14 February 1991 (14.02.91) pages 6, 8, 15, examples 2 and 3	1,2,4-8,15
X	AU,A,27538/92 (MEDVET SCIENCE PTY LTD) 15 April 1993 (15.04.93) page 3, figures 7 and 8	1,2,4-8,15
P,X	AU,A,56709/84 (GD SEARLE & CO.) 9 June 1994 (09.06.94) see pages 191-192, table 6	1,2,4-8,15

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1994 (15.11.94)	17 NOV 1994 (17.11.94)

Name and mailing address of the ISA/AU

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WODEN ACT 2606
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Authorized officer

J.H. CHAN

Telephone No. (06) 2832340

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
P,X	Proceedings of National Academy of Science USA, Vol. 91, issued 21 June 1994, "Specific human granulocyte-macrophage colony-stimulating factor antagonists", (T.R. HERCUS et al), pages 5838-5842, see column 2 page 5839.	1,2,3-12
P,X	The EMBO Journal, Vol. 12, No. 13 (1993), issued 15 December 1993, "Two distinct functional sites of human interleukin-4 are identified by variants impaired in either receptor binding or receptor activation" (N. KRUSE et al) pages 5121-5129, see table 1 pages 5122-5125.	1,2,4-8,18-31
P,X	European Journal of Biochemistry, Vol. 222, issued 1 June 1994, "Neutralizing monoclonal antibodies define two different functional sites in human interleukin-4", (REUSCH, P. et al), pages 491-499, see column 1 of page 492, tables 1, 2 and 3.	1,2,4-8,18-31
X	Proceedings of National Academy of Science USA, Vol. 85, issued October 1988, "Identification of specific residues of human interleukin-2 that effect binding to the 70-KDa subunit (p70) of the interleukin-2 receptor", pages 7709-7713, see tables 1 and 2.	1,2,4-8,16,17
P,X	Biochemistry 1994, Vol. 33, issued 31 May 1994, "Mutagenic Analysis of a Receptor Contact Site on Interleukin-2: Preparation of an IL-2 Analog with Increased Potency" (BERNDT WILLIAM G. et al), pages 6571-6577, see column 2 page 6571, tables 1 and 2.	1,2,4-8,16,17
X	The EMBO Journal, Vol. 11, No. 3, issued March 1992, "Residue 21 of human granulocyte-macrophage colony stimulating factor is critical for biological activity and for high but not low affinity binding", (A.F. LOPEZ et al), pages 909-916. See column 1 of page 910 to column 2 page 912.	1,2-12
A	Biochemical and Biophysical Research Communications, Vol. 159, No. 1, 1989, issued 28 February 1989, "Mutagenesis of human granulocyte colony stimulating factor", pages 103-111.	
A	Proceedings of National Academy of Science, Vol. 89, issued 1 December 1992, "A human interleukin-3 analog with increased biological and binding activities", (T. KUGO et al), pages 11842-11846.	
A	Biochimica et Biophysica Acta, Vol. 1041 (1990), "Theoretical conformational analysis of a family of alpha-helical immunocytokines (V.P. ZAV'YALOV et al), pages 178-185.	

B. FIELDS SEARCHED (supplemental box)

The following protein subsequences were searched in CA database in STN International:

HVNAIQ[HKR]ARRLLNL
ALVK[HKR]TLALLSTHRTL
NMI[HKR][DE]IITHL
NMI[DE][HKR]IITHL
NMI[HKR][HKR]IITHL
LLL[HKR]LQMIL
ITLQ[HKR]IIKTL
AYIL[HKR]GISALRK
GDQY[HKR]SVLMVSI
AGIL[HKR]INFLINKMQGD
NMLR[HKR]LADAFS
FLLKCL[HKR]QVRKI
YLLEAK[HKR]AENITTG

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	60690/90	US	5331148	WO	91/03029		
AU	27538/92	EP	609280	WO	93/07171		
AU	56709/94	AU	56125/94	WO	94/12638	WO	94/12639

END OF ANNEX

Ren/PCT 0589

CLAIMS

1 A peptide of 5-30 amino acids which peptide exhibits antagonistic activity directed against IL-6.

2 A peptide of 5-30 amino acids which peptide exhibits antagonistic activity directed against the α or β-chain of the IL-6 receptor.

3 A peptide of 5-30 amino acids which peptide exhibits antagonistic or agonistic IL-6 activity.

4 A peptide according to claim 1, 2 or 3 of 5-20 amino acids.

5 A peptide according to claim 4 of 5-12 amino acids.

6 A peptide according to claim 1, 2, 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNE-GKKMRCEWDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKFNPPHNLSVIN.

7 A peptide according to claim 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTT-KAVLLVRKFQNSPAED

8 A peptide composition, wherein at least two peptides according to any of claims 1-7 are chemically linked directly or via spacer molecules.

9 A peptide composition according to claim 8 wherein at least two peptides are linked with lysine.

10 A peptide composition wherein at least two peptides according to claim 1, 2, 3, 4, or 5 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

11 A peptide composition according to claim 8, 9 or 10 wherein at least four peptides are linked with branching oligolysines.

12 A mixture comprising peptides and/or peptide compositions according to any of claims 1-11.

13 Antibody specifically directed against a peptide or a peptide composition according to any of claims 1-11.

14 Anti-idiotypic antibody raised against an antibody according to claim 13.

15 A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to any of the above claims together with at least one suitable excipient for administration.

16 Use of a pharmaceutical preparation according to claim 15 in the treatment or prevention of an IL-6 related disease.

17 Use of a peptide, peptide composition or antibody according to anyone of claims 1-13 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

18 A diagnostic assay comprising a peptide or a peptide composition or an antibody according to anyone of claims 1-13.

19 Use of a diagnostic assay according to claim 18 to detect or diagnose IL-6 related disease in man or animals.

20 Use of a peptide according to claim 7 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 µg/ml when tested in vitro in a B9 cell bio assay.

21 Use of a peptide according to claim 20 in cell-culture.

22 A pharmaceutical preparation comprising a peptide according to claim 7 together with at least one suitable excipient for administration.

23 Use of a pharmaceutical preparation according to claim 22 for topical or intra-mammary application.

24 Use of a peptide, or peptide composition according to anyone of claims 1-12 for the manufacture of a medicament for topical or intra-mammary application.

09/202,[illegible]
63 Rec'd PCT/PTO 18 DEC 1998

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Loenardus Adrianus Maria Govardus van Leengoed et al.

Serial No.: PCT/NL97/00345

Filed: 19 June 1997

For: IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

Examiner:

Group Art Unit:

Attorney Docket No.: 3890

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number:
EL248225768US
Date of Deposit with USPS:
18 December 1998
Person making Deposit:
Jared Turner

PRELIMINARY AMENDMENT

Box
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above referenced application as indicated below prior to the calculation of the filing fee.

IN THE CLAIMS:

Cl

4. (Amended) A peptide according to claim 1 [, 2, or 3] of 5-20 amino acids.

6. (Amended) A peptide according to claim 1 [,2,3,4, or 5] having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM, QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKSEQATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

7. (Amended) A peptide according to claim 1 [3, 4, or 5] having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

8. (Amended) A peptide composition, wherein at least two peptides according to any of [claims 1-7] claim 1 are chemically linked directly or via spacer molecules.

10. (Amended) A peptide composition wherein at least two peptides according to claim 1 [,2, 3, 4, or 5] having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

11. (Amended) A peptide composition according to claim 8 [,9 or 10] wherein at least four peptides are linked with branching oligolysines.

12. (Amended) A mixture comprising peptides and/or peptide compositions according to [any of claims 1-11] claim 1.

13. (Amended) Antibody specifically directed against a peptide or a peptide composition according to [any of claims 1-11] claim 1.

15. (Amended) A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to [any of the above claims] claim 1 together with at least one suitable excipient for administration.

17. (Amended) Use of a peptide, peptide composition or antibody according to [anyone of claims 1-13] claim 1 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

18. (Amended) A diagnostic assay comprising a peptide [or a peptide composition or an antibody] according to [anyone of claims 1-13] claim 1.

24. (Amended) Use of a peptide [, or peptide composition according to anyone of claims 1-12] claim 1 for the manufacture of a medicament for topical or intra-mammary application.

Please add the following new claims:

25. A peptide according to claim 2 of 5-20 amino acids.

26. A peptide according to claim 25 of 5-12 amino acids.

27. A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

28. A peptide according to claim 2 of 5-20 amino acids.

29. A peptide according to claim 28 of 5-12 amino acids.

30. A peptide according to claim 2 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM, QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKSEQATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

31. A peptide according to claim 2 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence:
EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

32. A peptide composition, wherein at least two peptides according to claims 2 are chemically linked directly or via spacer molecules.

33. A peptide composition according to claim 32 wherein at least two peptides are linked with lysine.

34. A peptide composition according to claim 32 wherein at least four peptides are linked with branching oligolysines.

35. A peptide composition wherein at least two peptides according to claim 2 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

36. A mixture comprising peptides according to claim 2.

37. Antibody specifically directed against a peptide according to claim 2.

38. Anti-idiotypic antibody raised against an antibody according to claim 37.

39. A pharmaceutical preparation comprising a peptide according to claim 2 together with at least one suitable excipient for administration.

40. Use of a pharmaceutical preparation according to claim 39 in the treatment or prevention of an IL-6 related disease.

41. Use of a peptide according to claim 2 to clear extra-corporeal blood or blood products

from IL-6 or IL-6 receptor molecules.

42. A diagnostic assay comprising a peptide according to claim 2.

43. Use of a diagnostic assay according to claim 42 to detect or diagnose IL-6 related disease in man or animals.

44. Use of a peptide according to claim 31 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 micrograms/ml when tested in vitro in a B9 cell bio assay.

45. Use of a peptide according to claim 44 in a cell-culture.

46. A pharmaceutical preparation comprising a peptide according to claim 31 together with at least one suitable excipient for administration.

47. Use of a pharmaceutical preparation according to claim 6 for topical or intra-mammary application.

48. Use of a peptide according to claim 2 for the manufacture of a medicament for topical or intra-mammary application.

49. A peptide according to claim 3 of 5-20 amino acids.

50. A peptide according to claim 49 of 5-12 amino acids.

51. A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM, QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR,

NFTLKSEQATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

52. A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

53. A peptide composition, wherein at least two peptides according to claim 3 are chemically linked directly or via spacer molecules.

54. A peptide composition according to claim 53 wherein at least two peptides are linked with lysine.

55. A peptide composition wherein at least two peptides according to claim 3 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

56. A peptide composition according to claim 53 wherein at least four peptides are linked with branching oligolysines.

57. A peptide composition according to claim 55 wherein at least four peptides are linked with branching oligolysines.

58. A mixture comprising peptides and/or peptide compositions according to claim 3.

59. Antibody specifically directed against a peptide or a peptide composition according to claim 3.

60. Anti-idiotypic antibody raised against an antibody according to claim 59.

61. A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to claim 3 together with at least one suitable excipient for administration.

62. Use of a pharmaceutical preparation according to claim 61 in the treatment or prevention of an IL-6 related disease.

63. Use of a peptide, peptide composition or antibody according to claim 3 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

64. A diagnostic assay comprising a peptide according to claim 3.

65. Use of a diagnostic assay according to claim 64 to detect or diagnose IL-6 related disease in man or animals.

65. Use of a peptide according to claim 3 for the manufacture of a medicament for topical or intra-mammary application.

66. Use of a peptide according to claim 52 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 micrograms/ml when tested in vitro in a B9 cell bio assay.

67. Use of a peptide according to claim 66 in a cell-culture.

68. A pharmaceutical preparation comprising a peptide according to claim 52 together with at least one suitable excipient for administration.

69. Use of a pharmaceutical preparation according to claim 68 for topical or intra-mammary application.

70. Use of a peptide according to claim 3 for the manufacture of a medicament for topical

or intra-mammary application.

REMARKS

The Office is respectfully requested to enter the above amendment prior to the calculation of the filing fee.

Respectfully submitted,

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Date: December 18, 1998

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On page 15, line 27, after "PAED" please insert --(SEQ. ID. NO.: 11)--;

On page 16, line 5, after "SVIN" please insert --(SEQ. ID. NO.: 10)--;

On page 16, line 5, after "TSDH" please insert --(SEQ. ID. NO.: 9)--;

On page 16, line 5, after "FQGC" please insert --(SEQ. ID. NO.: 6)–.

IN THE FIGURES

On FIG. 1, after "SSLRALRQM" please insert --(SEQ. ID. NO.: 12)--;

On FIG. 2, after "PPANITV" please insert --(SEQ. ID. NO.: 13)--;

On FIG. 3, after "PHNLSVIN" please insert --(SEQ. ID. NO.: 14)–.

IN THE CLAIMS:

6. (Twice Amended) A peptide according to claim 1 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL (SEQ. ID. NO.: 2), ILRSFKEFLQSSLRALRQM (SEQ. ID. NO.: 3), QLSCFRKSPLSNVVC (SEQ. ID. NO.: 4), PRSTPSLTTKAVLLLVRKFQNS (SEQ. ID. NO.: 5), MCVASSVGSKFSKTQTFQGC (SEQ. ID. NO.: 6), PEKPKNLSCIVNEGKKMRCEWDGGR (SEQ. ID. NO.: 7), NFTLKSEQATHKFADCKAKRDTPTS (SEQ. ID. NO.: 8), WVEAENALGKVTSDH (SEQ. ID. NO.: 9), or PVYKVKPNPPHNLSVIN (SEQ. ID. NO.: 10).

7. (Twice Amended) A peptide according to claim 1 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED (SEQ. ID. NO.: 11).

10. (Twice Amended) A peptide composition wherein at least two peptides according to claim 1 having at least one string of 5 consecutive amino acids long in common with the amino acid

sequence RYILDGISALRK (SEQ. ID. NO.: 1) are linked with lysine.

B3 27. (Amended) A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED (SEQ. ID. NO.: 11).

B4 30. (Amended) A peptide according to claim 2 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL (SEQ. ID. NO.: 2), ILRSFKEFLQSSLRALRQM (SEQ. ID. NO.: 3), QLSCFRKSPLSNVVC (SEQ. ID. NO.: 4), PRSTPSLTTKAVLLLVRKFQNS (SEQ. ID. NO.: 5), MCVASSVGSKFSKTQTFQGC (SEQ. ID. NO.: 6), PEKPKNLSCIVNEGKKMRCEWDGGR (SEQ. ID. NO.: 7), NFTLKSEQATHKFADCKAKRDTPTS (SEQ. ID. NO.: 8), WVEAENALGKVTSDH (SEQ. ID. NO.: 9), or PVYKVKPNPPHNLSVIN (SEQ. ID. NO.: 10).

B5 31. (Amended) A peptide according to claim 2 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED (SEQ. ID. NO.: 11).

B6 35. (Amended) A peptide composition wherein at least two peptides according to claim 2 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK (SEQ. ID. NO.: 1) are linked with lysine.

B7 51. (Amended) A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL (SEQ. ID. NO.: 2), ILRSFKEFLQSSLRALRQM (SEQ. ID. NO.: 3), QLSCFRKSPLSNVVC (SEQ. ID. NO.: 4), PRSTPSLTTKAVLLLVRKFQNS (SEQ. ID. NO.: 5), MCVASSVGSKFSKTQTFQGC (SEQ. ID. NO.: 6), PEKPKNLSCIVNEGKKMRCEWDGGR (SEQ. ID. NO.: 7), NFTLKSEQATHKFADCKAKRDTPTS (SEQ. ID. NO.: 8), WVEAENALGKVTSDH (SEQ. ID. NO.: 9), or PVYKVKPNPPHNLSVIN (SEQ. ID. NO.: 10).

52. (Amended) A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED (SEQ. ID. NO.: 11).

55. (Amended) A peptide composition wherein at least two peptides according to claim 3 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK (SEQ. ID. NO.: 1) are linked with lysine.

REMARKS

The application is to be amended without prejudice or disclaimer as previously set forth. Primarily, the amendments are sought to conform the application to a form more consistent with Office practice.

The application is also to be amended to include a substitute SEQUENCE LISTING in conformity with 37 C.F.R. §§ 1.821-1.825. Submitted herewith is the substitute SEQUENCE LISTING, a copy of the substitute SEQUENCE LISTING in computer readable form, as well as a Statement Under 37 C.F.R. §§ 1.821(f) and 1.825.